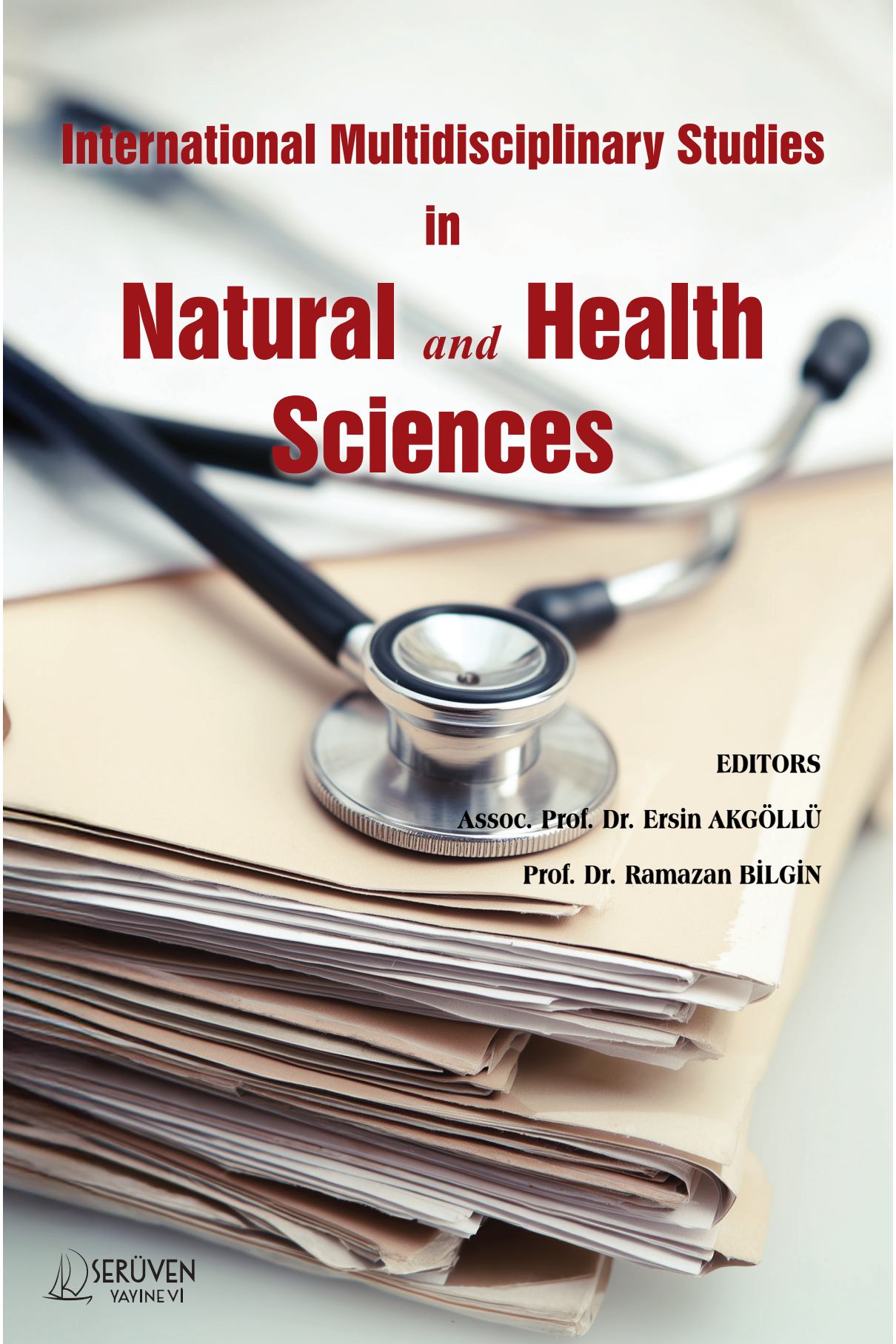
# International Multidisciplinary Studies in Natural *and* Health Sciences

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# Chapter 1

# INTEGRATIVE CONTRIBUTIONS OF BIOCHEMISTRY AND MOLECULAR GENETICS TO GENE THERAPY DEVELOPMENT

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## INTRODUCTION

Originally identified as part of the adaptive immune defence in bacteria, **Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)** were later repurposed as powerful tools for genome engineering (Wright et al., 2016). The CRISPR system typically comprises one or more nucleic acid-cleaving proteins and a guide RNA (gRNA) that directs the complex to specific nucleic acid sequences.

### Mechanistic Insights into CRISPR-Mediated DNA Cleavage

Gene-editing technologies have progressed across multiple generations. The first-generation wave featured zinc finger nucleases (ZFNs), followed by transcription activator-like effector nucleases (TALENs) in the second. The advent of CRISPR as a third-generation system significantly accelerated advancements in genome editing (Nemudryi et al., 2014). Functioning as a form of adaptive immunity, CRISPR allows bacteria and archaea to recognize and respond to prior viral infections. Within bacterial cells, CRISPR retains segments of foreign DNA, forming a molecular memory that enables precise targeting and cleavage of matching sequences through guide RNAs derived from these fragments. As a result, prokaryotic organisms utilize CRISPR machinery to degrade foreign genetic elements such as viral DNA or plasmids delivered by bacteriophages (Hsu et al., 2014; Park et al., 2024).

Compared to earlier gene-editing platforms such as ZFNs and TALENs, the CRISPR system offers key advantages, particularly in generating genome-scale targeting libraries with high efficiency. A distinct benefit lies in its multiplexing capability, wherein multiple guide RNAs can be employed concurrently to target various loci within a single cell (Cong et al., 2013; Mali et al., 2013). This facilitates simultaneous gene modifications or targeted deletions across specific genomic regions. For accurate DNA cleavage by the CRISPR-Cas system, the guide RNA must not only complement the target DNA sequence but also requires the presence of a specific protospacer adjacent motif (PAM) near the target site. This PAM requirement enhances target specificity and minimizes the risk of self-DNA cleavage. CRISPR systems are categorized into two broad classes and further subdivided into several types based on structural and functional characteristics. Among the numerous CRISPR systems identified in bacteria and archaea, a select few have been extensively adapted for genome editing in eukaryotic cells (Lee et al., 2020).

One of the most widely adopted tools is the Streptococcus pyogenes-derived CRISPR-Cas9 (SpCas9) system, which consists of a single Cas9 protein guided by a synthetic RNA molecule. This system exhibits

robust DNA cleavage activity and has been instrumental in advancing genome engineering in eukaryotes (Jinek et al., 2012). The Cas9 protein contains two primary lobes, a nuclease lobe and a recognition lobe, that work together bind the guide RNA and facilitate the formation of a stable RNA:DNA hybrid. The seed sequence, located within approximately 10 nucleotides upstream of the PAM site, is essential for effective binding. Although the PAM itself does not base-pair with the gRNA, it directly interacts with specific amino acid residues within the Cas9 protein, stabilizing the complex and enabling precise cleavage (Hsu et al., 2013).

**Use of the CRISPR System in Eukaryotes**

Following the delivery of CRISPR components, such as the SpCas9 protein, into eukaryotic cells, genome editing is achieved through a two-step process involving targeted DNA double-strand breaks (DSBs) and the subsequent activation of cellular DNA repair pathways. In the first phase, the Cas9 endonuclease, guided by a sequence-specific RNA molecule, introduces DSBs at predetermined genomic loci. This cleavage event serves as a trigger for the cell's intrinsic DNA repair machinery, which facilitates precise or error-prone editing at the break site (Park et al., 2024).

The outcomes of CRISPR-based genome editing typically include insertions, deletions, or the integration of exogenous DNA sequences, depending on the repair pathway engaged. In eukaryotic cells, DSBs are primarily repaired via non-homologous end joining (NHEJ), homologous recombination (HR), or microhomology-mediated end joining (MMEJ) (Sfeir & Symington, 2015). The utilization of these repair mechanisms can be context-dependent, with their activity influenced by cell type, the phase of the cell cycle, and DNA sequence context (Shrivastav et al., 2008). These pathways may operate either in competition or cooperatively, depending on the specific cellular environment. Among these, NHEJ is the most prevalent and functions throughout all stages of the cell cycle. The NHEJ process comprises four major steps: a) Recognition of DNA ends b) Bridging of the broken DNA strands and formation of a repair complex (synapsis) c) Processing of the DNA termini d) Ligation of the DNA ends (Deriano & Roth, 2013). Critical protein assemblies in the non-homologous end joining (NHEJ) pathway include Ku70/80, MRE11, the Artemis-DNA-PKcs complex, and the XLF-XRCC4-DNA ligase IV complex. While NHEJ is a rapid and efficient repair mechanism, it is prone to errors because it operates without the need for a homologous DNA template. This absence often leads to insertions or deletions (indels) at the site of repair, which can cause frameshift mutations or introduce premature stop codons if these changes occur within coding sequences, ultimately result-

ing in gene disruption or knockout (Deriano & Roth, 2013; Lieber, 2010).

Homologous recombination (HR) is predominantly active during the S and G2 phases of the eukaryotic cell cycle, when a sister chromatid is available to serve as a repair template. The process is initiated by a coordinated network of DNA repair proteins, including Rad51, Rad52, Rad54, BRCA2, and replication protein A (RPA). HR unfolds through a series of tightly regulated steps:

a) Recognition and resection of DNA ends, generating 3' single-stranded overhangs b) Strand invasion and homologous pairing, mediated primarily by Rad51 c) Extension of the DNA heteroduplex and branch migration, allowing stable strand exchange d) Resolution of Holliday junctions, restoring intact DNA duplexes (Krejci et al., 2012). Unlike error-prone repair pathways, HR utilizes the homologous sequence on the sister chromatid as a template, ensuring high-fidelity repair of double-strand breaks without altering the original DNA sequence. This mechanism is therefore critical for maintaining genomic integrity during cell proliferation (Heyer et al., 2010).

Microhomology-mediated end joining (MMEJ) represents an alternative, error-prone form of the non-homologous end joining (NHEJ) pathway and is predominantly active during the G1 and early S phases of the cell cycle. Unlike classical NHEJ (c-NHEJ), which ligates DNA ends without the need for homology, MMEJ relies on the presence of short microhomologous sequences, typically around 5 to 25 base pairs, flanking the double-strand break (DSB) site to facilitate repair (Sakuma et al., 2016). The repair process begins with the recruitment of specific protein factors, including PARP1, MRE11, CtIP, and the XRCC1:DNA ligase I/III complex, to the DNA lesion. These proteins promote the resection of DNA ends and expose the microhomologous regions. Alignment of these sequences allows for annealing, followed by trimming of the overhangs and ligation. Due to the nature of this process, MMEJ often results in deletions of the intervening DNA sequence between the microhomologies, contributing to its inherently mutagenic character. As such, while MMEJ provides a backup mechanism for DSB repair, its error-prone outcomes can lead to genomic instability if not tightly regulated (Bae et al., 2014).

CRISPR-based genome editing harnesses the cell's endogenous DNA repair mechanisms to achieve targeted genetic modifications. By inducing site-specific double-strand breaks (DSBs), the technology enables either the disruption of genes through error-prone repair or the precise integration of exogenous DNA sequences via homology-directed repair. However, a key challenge arises from the fact that multiple DNA repair pathways such as NHEJ, MMEJ, and HR, can be simultaneously activat-

ed in response to a DSB. These pathways often function in a competitive or overlapping manner, making the outcome of the repair process unpredictable. As a result, the exact nature of sequence alterations introduced at the target site can vary, complicating efforts to achieve consistent and precise genome edits (Heyer et al., 2010; Shrivastav et al., 2008).

**Gene Therapy Approaches with CRISPR-Induced Double-Stranded DNA Breaks in Genetic Diseases**

The ability of CRISPR technology to induce precise double-strand breaks (DSBs) at designated loci within the eukaryotic genome has substantially expanded the scope and precision of genome editing strategies (Mali et al., 2013). This approach has been successfully adapted for the development of gene therapies targeting pathogenic DNA variants identified through clinical genomic analyses, offering promising avenues for the treatment or amelioration of genetic diseases. Numerous studies have demonstrated the therapeutic potential of CRISPR-based interventions, where targeted DSBs facilitate site-specific genome modifications (Cho et al., 2013). These include the correction of disease-causing mutations, disruption of harmful gene expression, and insertion of therapeutic sequences, collectively underscoring CRISPR's transformative role in precision medicine. Genetic diseases that are being tested using CRISPR genome editing through double-stranded DNA break are Gaucher disease, Wolfram syndrome, Myeloproliferative neoplasm, Huntington disease, cancer, Sickle cell disease, Transthyretin amyloidosis, Corneal dystrophy, Retinitis pigmentosa. Several of these will be briefly outlined in the following section (Park et al., 2024).

**Gaucher Disease**

Gaucher disease type 1 (GD1), the most prevalent variant, stems from mutations in the GBA gene that impair glucocerebrosidase (GCase) function, leading to glycolipid buildup predominantly in macrophages due to their high demand for glycolipid turnover (Stirnemann et al., 2017). Currently, GD treatment relies on two main approaches: lifelong enzyme replacement therapy (ERT) involving intravenous infusion of recombinant GCase, and substrate reduction therapy (SRT) using orally administered glucosylceramide synthase inhibitors. These therapies are non-curative, decline symptoms, high-cost, and require using lifelong. An alternative strategy involves genetic correction of the patient's own hematopoietic stem and progenitor cells (HSPCs) to restore endogenous GCase expression. This approach holds the potential to provide a one-time, curative treatment by reconstituting a macrophage population capable of proper glycolipid degradation. For this reason, Scharenberg et al. (2020) em-

ployed a CRISPR-Cas9 ribonucleoprotein (RNP)/AAV6-based platform to achieve targeted integration of a therapeutic GCase transgene into the CCR5 safe harbor locus in human HSPCs. By utilizing a lineage-specific promoter active in the monocyte/macrophage lineage, the researchers ensured GCase expression in relevant cell types while minimizing off-target expression in hematopoietic progenitors. These engineered HSPCs demonstrated stable engraftment and multi-lineage differentiation, including the generation of macrophages expressing supraphysiologic levels of GCase in vivo. This approach represents a promising step toward developing a durable and potentially curative gene therapy for GD1, overcoming the limitations of current treatment modalities (Scharenberg et al., 2020). In a key study by Scharenberg et al. (2020), the GCase gene was integrated into human blood stem cells using the CRISPR-Cas9 + AAV6 platform. Moreover, these edited cells were transplanted into mouse models and developed into functional macrophages, producing high levels of GCase. However, this work is preclinical and has not yet been published as a formal clinical trial in humans.

**Cancer**

Cai et al., (2022) reported that a cell-selective and enzyme-responsive genome editing system, termed enzyme-inducible CRISPR (eiCRISPR), was evaluated in HEK 293/GFP and HeLa/GFP cells, derived from human embryonic kidney and cervical cancer cell lines, respectively. The system consists of Cas9 protein, a self-inhibited single-guide RNA (bsgRNA), and a chemically caged deoxyribozyme (DNAzyme). The DNAzyme is modified to remain inactive under normal conditions, preventing cleavage of the bsgRNA. In cells overexpressing disease-associated enzymes such as NAD(P)H:quinone oxidoreductase 1 (NQO1), the caging group is removed, activating the DNAzyme. This leads to bsgRNA activation and targeted genome editing specifically in enzyme-rich tumor cells. Additionally, the eiCRISPR system was delivered in vivo using biodegradable lipid nanoparticles in a mouse tumor xenograft model, enabling selective editing of the HPV-18 E6 gene. This strategy demonstrates the feasibility of using endogenous enzymatic activity to control genome editing for targeted gene therapy (Cai et al., 2022). This study is a pre-clinical study and has not yet been tested in humans.

**Huntington's Disease (HD)**

HD is a prototypical example of a genetic disorder caused by a dominant mutation that exerts toxicity through a mutant protein. In HD, an expanded CAG trinucleotide repeat in the HTT gene produces a huntingtin protein with an excessively elongated polyglutamine stretch (Orr &

Zoghbi, 2007). When the CAG repeat exceeds 35 units, it causes fully penetrant clinical symptoms, including motor dysfunction, cognitive impairment, and psychiatric disturbances, regardless of the surrounding genetic haplotype. The understanding that disease pathogenesis in HD is driven by the presence of the mutant protein has led to the development of gene-silencing strategies as potential therapies (Bates et al., 2015; Lee et al., 2012). Notably, individuals with a balanced chromosomal translocation resulting in loss of one HTT allele do not exhibit HD symptoms, indicating that HD is not caused by HTT haploinsufficiency and that a single functional allele is sufficient for normal cellular function. These findings support the rationale for permanently and selectively inactivating the mutant HTT allele as a targeted therapeutic strategy and suggest broader applicability to other dominant genetic diseases. It is introduced a novel CRISPR/Cas9-based approach for allele-specific genome editing targeting single nucleotide polymorphisms (SNPs) associated with dominant genetic disorders. The method was designed to selectively and permanently inactivate mutant alleles without affecting the wild-type counterpart, offering a potential therapeutic strategy for autosomal dominant diseases (Bates et al., 2015; Shin et al., 2016). Using Huntington's disease (HD) as a model, caused by a toxic gain-of-function CAG repeat expansion in the HTT gene, they developed a haplotype-specific CRISPR/Cas9 system exploiting PAM-altering SNPs. By designing two guide RNAs owing to its capacity to create large genomic deletions between the two targeted sites, that recognize PAM sequences unique to the mutant allele, they achieved a precise excision of a ~44 kb genomic region encompassing the promoter, transcription start site, and expanded CAG repeats. This led to complete and selective inactivation of the mutant HTT allele, abolishing expression of the mutant mRNA and protein, while leaving the wild-type allele intact. This approach demonstrates high allele specificity and has broad potential for precision medicine applications targeting diverse gain-of-function mutations in dominantly inherited conditions (Shin et al., 2016). CRISPR-based therapies for Huntington's disease (HD) have not yet become widely applicable to humans clinically, but significant progress has been made in the field and studies are currently in the preclinical (in vitro and in vivo) stages.

**Sickle Cell Disease**

Transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) are among the most widespread monogenic disorders worldwide, with an estimated annual incidence of around 60,000 cases for TDT and 300,000 for SCD (Frangoul et al., 2021). Both conditions originate from mutations in the β-globin gene (HBB). In TDT, such mutations lead to either a partial ($\beta^{+}$) or complete ($\beta^{0}$) deficiency in β-globin production,

disrupting the balance between α- and β-globin chains and ultimately resulting in ineffective erythropoiesis (Pasricha & Drakesmith, 2018; Shah et al., 2019). Conversely, SCD is caused by a single nucleotide substitution in HBB that leads to the replacement of glutamic acid with valine, producing sickle hemoglobin (HbS). Upon deoxygenation, HbS polymerizes, inducing red blood cell deformation, chronic hemolysis, anemia, painful vaso-occlusive crises, progressive organ damage, and ultimately shortened lifespan (Pasricha & Drakesmith, 2018; Shah et al., 2019).

Genome-wide association studies (GWAS) have uncovered specific single-nucleotide polymorphisms (SNPs) linked to elevated levels of fetal hemoglobin (HbF), a hemoglobin variant composed of two alpha and two gamma chains, in adult individuals (Bauer et al., 2013). Notably, several of these SNPs reside within the BCL11A locus on chromosome 2 and correlate with a milder clinical presentation of both TDT and SCD (Uda et al., 2008). BCL11A encodes a zinc finger transcription factor that acts as a repressor of γ-globin gene expression in erythroid cells. SNPs associated with elevated HbF are situated within an erythroid-specific enhancer region of BCL11A; these variants lead to reduced BCL11A expression and, in turn, enhanced γ-globin transcription and HbF production. It has reported that the therapeutic potential of CRISPR-Cas9 gene editing by targeting the erythroid-specific enhancer of the *BCL11A* gene in $CD34^+$ hematopoietic stem and progenitor cells (HSPCs). Electroporation of these cells with CRISPR-Cas9 resulted in efficient editing, with approximately 80% of alleles modified at the target site and no detectable off-target effects. Following myeloablative conditioning, two patients (one diagnosed with TDT and the other with SCD) received autologous $CD34^+$ HSPCs edited at the same *BCL11A* enhancer region. Over a year post-infusion, both patients exhibited sustained high levels of allelic modification in peripheral blood and bone marrow, marked elevations in HbF expression, independence from blood transfusions, and, in the case of the SCD patient, complete resolution of vaso-occlusive crises (Frangoul et al., 2021). This method was developed by CRISPR Therapeutics & Vertex Pharmaceuticals and currently applied to TDT and SCD patients. As mentioned above, the hematopoietic stem cells (HSC) taken from the patient are transformed into cells with reactivated HbF expression by cutting the erythroid-specific enhancer region of BCL11A with CRISPR/Cas9 in the laboratory and treated by returning them to the patient via bone marrow transplantation.

## CRISPR-Induced Base and Prime Editing Approaches in Gene Editing

Base editing technology has emerged as a precise gene editing strategy that enables the substitution of a single nucleotide at a defined genomic

locus by coupling a base-modifying enzyme with a re-engineered CRISPR system that introduces single-strand nicks instead of double-strand breaks (DSBs) (Gaudelli et al., 2017; Komor et al., 2016). These systems are generally capable of altering bases within a narrow editing window, typically around 10 nucleotides, facilitating targeted base conversions such as cytosine to thymine or adenine to guanine. However, the editable region can be modulated to extend or restrict beyond this range, depending on the editing objective. By eliminating the need for DSBs, base editing provides a safer and more efficient alternative for precise genome modifications (Komor et al., 2016; Nishida et al., 2016). The initial cytidine base editors (CBEs) employed either a catalytically impaired SpCas9 nickase (nCas9) or a catalytically inactive SpCas9 (dCas9), fused to a cytidine deaminase. A D10A mutation in nCas9 selectively inactivates the RuvC nuclease domain, allowing site-specific nicking of the target DNA strand via the HNH domain, while dCas9 carries both D10A and H840A mutations, rendering the protein nuclease inactive. CBEs catalyze the deamination of cytidine to uridine in the target sequence, which is then recognized as thymine during replication or repair, leading to a cytosine-to-thymine substitution at the DNA level (Komor et al., 2016; Nishida et al., 2016). Other method, prime editing, like base editing in that it avoids the generation of double-stranded DNA breaks (DSBs), operates through a distinct molecular mechanism. Unlike base editors, which rely on deaminase enzymes to induce base conversions, prime editing utilizes a reverse transcriptase (RT) to incorporate new genetic information. The system employs a prime editing guide RNA (pegRNA), which serves a dual role: it functions both as a template for reverse transcription and as a guide RNA to direct the editing complex to the target genomic sequence (Lin et al., 2020; Liu et al., 2020).

Prime editing, initially developed using SpCas9, operates through a three-step process: single-strand DNA cleavage, reverse transcription–mediated DNA synthesis, and genomic incorporation via DNA repair. The modified SpCas9 nickase (H840A) introduces a nick at the target site, allowing the pegRNA to hybridize with the exposed DNA strand. A fused Moloney murine leukemia virus (M-MLV) reverse transcriptase extends the DNA using the pegRNA as a template, generating the desired sequence (Deriano & Roth, 2013). This newly synthesized strand is then integrated into the genome through endogenous repair pathways. The original version (PE1) demonstrated limited editing efficiency, which was improved in PE2 through reverse transcriptase optimization. PE3 further enhanced outcomes by introducing an additional nick on the non-edited strand to favor incorporation of the edited sequence. PE4 and PE5 increased efficiency by transient inhibition of mismatch repair (MMR). Subsequent re-

finements involved using catalytically inactive sgRNAs (dsgRNAs) and chromatin-modulating peptides to facilitate Cas9 binding and potentially enhance editing through chromatin remodeling (Park et al., 2024).

**CRISPR-Mediated Base and Prime Editing Techniques Targeting Genetic Disorders**

Base editing enables the accurate installation of single-nucleotide changes in genomic DNA without the need for double-stranded breaks or homology-directed repair, mechanisms that are typically inefficient in non-dividing cells. Genetic diseases that are being tested using CRISPR-mediated base and prime editing without double-stranded DNA break are spinal muscular atrophy (SMA), late-onset Alzheimer's disease, Duchenne muscular dystrophy, β-thalassemia, sickle-cell anemia, hereditary haemochromatosis, hypertrophic cardiomyopathy, retinitis pigmentosa (Park et al., 2024). Several of these will be briefly outlined in the following section.

**Late-Onset Alzheimer's Disease (AD)**

AD is a common neurodegenerative disorder, and patients exhibit severe motor and cognitive decline, including aphasia, apraxia, and incontinence, and are often bedridden. Hallucinations, circadian rhythm disruption, and behavioural symptoms occur in a substantial number of patients (Krishnamurthy et al., 2024). One of the factors affecting the disease is APOE4 apolipoprotein. It promotes amyloid-β aggregation and tau hyperphosphorylation, both hallmarks of AD pathology (Huang, 2006). A study demonstrated the use of the BE3 cytosine base editor to amend disease-related point mutations in the APOE4 gene, which is linked to late-onset Alzheimer's disease. In mouse astrocytes, BE3 delivery effectively reversed the nucleotide alterations responsible for the C158R and Y163C variants, reaching correction rates as high as 74.9% (Komor et al., 2016). Although APOE4 is a well-known risk factor for AD, CRISPR- or base editing–based human clinical trials targeting these specific mutations (C158R and Y163C) are currently unavailable. Correction of these mutations has so far been performed primarily in cell cultures and animal models. They are being tested in animal and cell culture models.

**Duchenne muscular dystrophy (DMD)**

Therapeutic use of adenine base editing (ABE) was validated in a DMD mouse model carrying a nonsense mutation in the Dmd gene. In their study, Ryu et al. (2018) showed that intramuscular administration of two trans-splicing AAV vectors encoding ABE7.10 into the tibialis anterior muscle enabled accurate A-to-G nucleotide conversions, effective-

ly restoring the premature stop codon to a glutamine codon (Ryu et al., 2018). The first human CRISPR application was approved by the US FDA in 2023. CRISPR components are delivered to the muscles with AAV (adeno-associated virus). However, these applications are still in the phase 1/2 safety and efficacy stage.

**Hereditary Haemochromatosis**

Adenine base editing (ABE) has also been utilized to correct pathogenic single-base mutations associated with hereditary hemochromatosis. Hereditary hemochromatosis is primarily caused by a G>A mutation (c.845G>A; p.Cys282Tyr) in the HFE gene, leading to protein misfolding and impaired interaction with transferrin receptors. This defect disrupts iron homeostasis, resulting in excessive intestinal iron absorption and accumulation of iron in organs, particularly in the liver, heart, and pancreas (Feder et al., 1998). Clinical consequences include cirrhosis, diabetes, and cardiomyopathy. Current treatment relies on lifelong phlebotomy and iron chelation therapy (Rovai et al., 2022). A single administration of a split AAV-based ABE7.10 system in a mouse model of hereditary hemochromatosis corrected over 10% of the HFE mutation and ameliorated abnormal iron metabolism in the liver. Studies in this area are still being conducted at the preclinical level, that is, in laboratory environments such as animal models and cell cultures.

**Spinal Muscular Atrophy (SMA)**

SMA is a degenerative disorder of motor neurons and represents the most common genetic cause of infant mortality. The disease arises from a homozygous deletion or mutation in the survival motor neuron 1 (SMN1) gene, which is critical for motor neuron viability. It has been demonstrated that the development of adenine base editor (ABE)-mediated gene therapy for SMA, a neurodegenerative disorder caused by the deficiency of survival motor neuron (SMN) protein (Boda et al., 2004; Rochette et al., 2001). Efficient A-to-G base conversion within the SMN2 gene was achieved in both cell models and an SMA mouse model that expressed the truncated, non-functional SMNΔ7 protein. Remarkably, a single intracerebroventricular injection of AAV9 encoding the ABE resulted in an average editing efficiency of 87% in the SMN2 gene in vivo. These findings suggest that a one-time administration of ABE-based gene therapy holds promise as a curative approach for SMA (Arbab et al., 2023).

**SMA Treatment Using AAV9-SMN1 and Alternative Methods**

Although the nearly identical SMN2 gene (>99.9% sequence homology) can partly compensate for SMN1 deficiency, a single synonymous

nucleotide change (C to T at position 6 of exon 7) leads to the exclusion of exon 7 from most SMN2 transcripts (mRNA) (Boda et al., 2004; Rochette et al., 2001). This results in the production of a truncated and unstable SMNΔ7 protein, ultimately leading to insufficient levels of functional SMN, progressive motor neuron degeneration, muscle weakness, and fatal outcomes (Cho & Dreyfuss, 2010). Without intervention, individuals with the most severe form (type I) have a median survival of approximately six months (Cobben et al., 2008). Currently, three landmark therapies have significantly improved outcomes in SMA by enhancing SMN protein expression. Among these, the antisense oligonucleotide **nusinersen** (Spinraza) and the orally administered small molecule **risdiplam** (Evrysdi) function by promoting exon 7 inclusion during SMN2 pre-mRNA splicing, thereby approximately doubling SMN protein levels (Baranello et al., 2021; Chiriboga et al., 2016). The mechanisms of these two drugs are as follows, respectively; Nusinersen (Spinraza) is an antisense oligonucleotide designed to ensure that exon 7 of the SMN2 gene is properly spliced. This drug binds to an intronic region immediately adjacent to exon 7 called ISS-N1 (Intronic Splicing Silencer N1). Some cellular proteins that normally bind to this region (such as hnRNP A1) cause exon 7 to be skipped. However, Nusinersen binds to this region, preventing these suppressor proteins from binding. Thus, the cell's splicing system includes exon 7 rather than excludes it, allowing more full-length, functional SMN protein to be produced via the SMN2 gene (Lorson et al., 1999; Singh et al., 2006). Risdiplam interacts with the boundaries of exon 7 in the SMN2 pre-mRNA and the intronic sequences immediately surrounding it. This interaction promotes the spliceosome to recognize and splice exon 7 more efficiently. It is not known exactly which regions it interacts with; however, it allows the synthesis of fully functional SMN2 protein (Ratni et al., 2018).

The third drug used, **Zolgensma** (onasemnogene abeparvovec-xioi), is given to patients via AAV9 vectors. Adeno-associated virus (AAV) vectors, such as those carrying the SMN1 gene that is synthesized in vitro as only complementary DNA (cDNA) consisting of exons, for spinal muscular atrophy (SMA) therapy, are typically produced in HEK293 cells using a triple plasmid transfection system. This includes: (1) a transgene plasmid encoding the therapeutic gene cassette (e.g., *SMN1* or *SMN2* with a promoter), (2) a Rep/Cap plasmid encoding the replication and capsid proteins specific to the desired AAV serotype (e.g., AAV9), and (3) a helper plasmid providing essential adenoviral genes for AAV replication (Wright, 2008). After 48–72 hours of incubation, the cells are harvested and lysed, both enzymatically and mechanically, to release intracellular AAV particles. The crude lysate is then purified through multiple steps,

such as iodixanol gradient ultracentrifugation or ion-exchange chromatography, to separate full capsids from empty ones and eliminate host cell contaminants. The final product is concentrated, formulated in a physiological buffer with stabilizers, sterile-filtered, and subjected to rigorous quality control testing, including assays for identity, potency, sterility, and endotoxin levels, in accordance with Good Manufacturing Practice (GMP) standards. Only after meeting all these criteria is the AAV vector deemed suitable for clinical administration to SMA patients (Wright, 2020).

**Conclusion**

Research in biochemistry, molecular biology, genetics, and genetic engineering has rapidly advanced the search for treatments of genetic diseases. CRISPR technologies, including base editing and prime editing, have enabled not only the correction of heritable mutations and single nucleotide polymorphisms but also the precise excision or replacement of longer nucleotide sequences, which has become feasible in vivo. It is being tested in the treatment of diseases associated with approximately 238 genetic disorders, especially by delivering healthy genes designed in vitro via AAV vectors. The successful use of AAV9 vectors carrying the SMN1 gene to treat spinal muscular atrophy (SMA) patients exemplifies this progress. This breakthrough has opened a promising new avenue for the treatment of genetic diseases. With the rapid advancement of technology, it is increasingly expected that future generations will build upon current research to develop gene therapies for a wide range of diseases. Alongside these hopeful developments, I believe that in the coming decades, treatments for numerous conditions, including cancer, will improve significantly, ultimately leading to increased human lifespan.

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Chapter 2

# CHRONIC LYMPHOCYTIC LEUKEMIA: MODERN INSIGHTS AND PERSONALIZED TREATMENT STRATEGIES

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## INTRODUCTION

Chronic lymphocytic leukaemia (CLL) is a malignancy of mature $CD5^+$ B lymphocytes (Hallek, 2019). Some patients with CLL remain asymptomatic for years, whereas others experience rapid disease progression that necessitates early treatment. The pathogenesis of CLL involves both intrinsic genetic mutations and extrinsic microenvironmental factors (Hernández et al., 2021).

In recent years, advancements in technologies such as sequencing and flow cytometry have significantly transformed the management of CLL. Rather than delaying treatment until clinical symptoms appear, therapeutic strategies are now increasingly guided by genetic and molecular profiling, leading to more personalized and targeted interventions (Burger & O'Brien, 2018).

### 1. Pathophysiology and Genetic Landscape

CLL originates from mature B lymphocytes that typically express CD5, CD19, CD23, and low levels of surface immunoglobulins. CLL can be classified into two subtypes according to the immunoglobulin heavy-chain variable region (IGHV):

➢Mutated IGHV (M-CLL): Generally associated with a more indolent clinical course.

➢Unmutated IGHV (U-CLL): Typically presents with more aggressive disease and a poorer prognosis (Damle et al., 1999).

➢Common chromosomal abnormalities in CLL include:

➢Deletion of 13q14 (~55% of cases)

➢Deletion of 11q22–23, involving the *ATM* gene

➢Trisomy 12

➢Deletion of 17p13, affecting the *TP53* gene

These genetic alterations serve not only as prognostic indicators but also guide therapeutic decision-making. Resistance to conventional chemoimmunotherapy is commonly observed in patients carrying TP53 mutations or 17p deletions, necessitating the use of targeted therapies such as Bruton's tyrosine kinase (BTK) or BCL-2 inhibitors (Wierda et al., 2020).

Additionally, scientists have explored the role of specific single nucleotide polymorphisms (SNPs) in CLL development and treatment outcomes.

One such polymorphism is the C677T variant in the methylenetetrahydrofolate reductase (MTHFR) gene, which leads to decreased enzyme activity. This genetic variation may influence DNA methylation and potentially alter the response to antifolate therapies (Frosst et al., 1995; Kim, 2007).

## 2. Clinical Features and Diagnosis

CLL is often discovered incidentally during routine blood work. The hallmark finding is a persistently elevated lymphocyte count exceeding $5\times10^9$/L for more than three months. Diagnosis is confirmed using flow cytometry, which detects a characteristic immunophenotype: CD5+, CD19+, CD23+, with weak expression of CD20 and surface immunoglobulins (Hallek et al., 2019).

Patients may remain symptom-free for a long time. However, when symptoms do occur, they commonly include:

- Fatigue
- Night sweats
- Unintentional weight loss
- Frequent infections due to weakened immunity

Physical examination may reveal lymphadenopathy, splenomegaly, or hepatomegaly. In certain patients, CLL can undergo transformation into an aggressive large B-cell lymphoma, known as Richter's transformation, which typically indicates an unfavourable clinical outcome (Tadmor et al., 2021).

Immune dysregulation is a common feature of CLL, with some patients developing autoimmune cytopenias such as autoimmune hemolytic anemia (AIHA) or immune thrombocytopenia (ITP), characterized by immune-mediated destruction of erythrocytes or platelets. These complications contribute to disease-related morbidity and often necessitate immunosuppressive therapy for effective management (Vitale et al., 2020).

## 3. Staging and Prognostic Scoring in CLL

### 3.1 Classical Staging Systems

#### 3.1.1 Rai Staging System

In CLL, staging is essential for assessing disease severity and guiding clinical management. The Rai and Binet systems remain the most widely used traditional approaches, especially in the United States and Europe, respectively.

The Rai system categorizes patients from Stage 0 to Stage IV based on lymphocytosis, lymphadenopathy, organomegaly, anemia, and thrombocytopenia. Lower stages (0–II) represent low to intermediate risk, while Stages III and IV are considered high risk due to the presence of anemia (hemoglobin <11 g/dL) or thrombocytopenia (platelets <100,000/μL) (Hallek et al., 2008).

### 3.1.2 Binet Staging System

The Binet system divides patients into three groups (A–C) according to the number of affected lymphoid areas and the presence of anemia or thrombocytopenia. Stage A involves fewer than three lymphoid areas without cytopenias, Stage B includes three or more areas without anemia or thrombocytopenia, and Stage C reflects cytopenias regardless of lymph node involvement (Hallek et al., 2008).

## 3.2 Modern Prognostic Tools

### 3.2.1 CLL International Prognostic Index (CLL-IPI)

This scoring system integrates:

- **TP53 mutation or deletion**
- **IGHV mutation status**
- **β2-microglobulin levels**
- **Clinical stage** (Rai or Binet)
- **Patient age**

Patients are classified into distinct prognostic risk categories, ranging from low to very high, which inform treatment selection and provide critical estimates of overall survival (OS) and progression-free survival (PFS) (Wang et al., 2021).

### 3.2.2 Next-Generation Sequencing (NGS)

NGS allows for the detection of mutations in key genes, including:

- **NOTCH1**
- **SF3B1**
- **BIRC3**

These molecular alterations provide deeper insight into prognosis and therapeutic planning (Rossi et al., 2012).

### 3.2.3 Fluorescence in Situ Hybridization (FISH)

FISH is critical for identifying chromosomal abnormalities such as:

- **del(13q)**
- **del(11q)**
- **trisomy 12**
- **del(17p)**

These cytogenetic markers significantly influence treatment selection, particularly regarding suitability for targeted therapies (Döhner et al., 2000).

## 4. Conventional Treatment Modalities

The therapeutic landscape of CLL initially centered on alkylating agents such as chlorambucil. As treatment strategies advanced, purine analogs like fludarabine were introduced, ultimately paving the way for combination chemoimmunotherapy (CIT) regimens. Among these, the FCR protocol, comprising fludarabine, cyclophosphamide, and rituximab, has emerged as a well-established standard. FCR continues to induce long-lasting remissions, particularly in biologically favorable subgroups, such as patients with mutated IGHV genes and functional TP53, thereby retaining its relevance in the management of younger, fit individuals (Eichhorst et al., 2021).

For older individuals or those with significant comorbidities, the bendamustine plus rituximab (BR) regimen is often preferred due to its improved tolerability profile. However, both FCR and BR can induce profound immunosuppression, increasing the risk of infections and cytopenias.

Importantly, patients with TP53 mutations or 17p deletions derive minimal to no benefit from conventional CIT. In this high-risk group, chemoimmunotherapy is no longer considered appropriate, and alternative treatment strategies are warranted (Fischer et al., 2019).

For patients diagnosed at early disease stages (e.g., Rai stage 0 or Binet stage A) who exhibit no signs of progression or symptoms, the current standard approach remains “watchful waiting”. In such cases, treatment initiation is deferred until clear clinical progression or active disease is observed, as defined by the iwCLL criteria (Hallek & Cheson et al., 2018).

## 5. Targeted Therapies

CLL care has advanced significantly with the use of therapies aimed at specific molecular mechanisms.

### 5.1 BTK Inhibitors

Ibrutinib, an oral BTK inhibitor, blocks B-cell receptor signalling and inhibits the survival of leukemic cells. It has indicated significant improvements in survival in both treatment-naïve and relapsed/refractory CLL patients. Long-term follow-up data from a phase 2 study confirmed sustained efficacy, with a median progression-free survival of approximately 7.2 years and overall survival rates of 73.8% at nearly 10 years (Ahn et al., 2018).

Second-generation BTK inhibitors, such as acalabrutinib and zanubrutinib, have demonstrated comparable efficacy to ibrutinib in the treatment of CLL. Notably, these agents exhibit improved safety profiles, with reduced incidences of cardiovascular and bleeding-related adverse events. Clinical trials, including the ELEVATE-RR and ALPINE studies, have reinforced their role as effective and better-tolerated alternatives in both treatment-naive and relapsed/refractory CLL patients (Tam et al., 2020).

### 5.2 BCL-2 Inhibitors

Venetoclax targets the anti-apoptotic protein BCL-2, restoring the cell's natural death mechanisms. When combined with obinutuzumab, it can induce deep responses, often with undetectable minimal residual disease (uMRD) (Fischer et al., 2019).

The combination of venetoclax and rituximab has indicated high efficacy in relapsed or refractory CLL patients. Notably, these regimens are often time-limited, typically administered for six to twelve months, which can reduce long-term toxicity and improve patient quality of life. Recent follow-up data from the MURANO trial confirmed sustained progression-free survival benefits with fixed-duration venetoclax-rituximab therapy (Seymour et al., 2018; Kater et al., 2020).

### 5.3 PI3K Inhibitors

The drugs idelalisib and duvelisib inhibit the PI3Kδ pathway and are approved for use in relapsed or refractory CLL. While effective, their clinical utility is limited by immune-mediated adverse effects, including colitis, hepatotoxicity, and pneumonitis. Recent studies have highlighted the need for careful monitoring and management of these toxicities to optimize patient outcomes (Ghia et al., 2014)

### 5.4 Anti-CD20 Monoclonal Antibodies

In addition to rituximab, newer generation anti-CD20 monoclonal antibodies have been developed to enhance therapeutic efficacy. These include:

➢ **Obinutuzumab:** A glycoengineered antibody designed to improve immune cell-mediated cytotoxicity.

➢ **Ofatumumab**: A fully human monoclonal antibody targeting a distinct epitope on the CD20 antigen.

These agents are frequently administered in combination with venetoclax or chlorambucil, particularly in elderly or frail patients who may not tolerate intensive chemotherapy regimens. (Al-Sawaf et al., 2024)

## 6. MTHFR Polymorphisms and Their Role in CLL

The MTHFR enzyme plays a critical role in folate metabolism, which is essential for DNA synthesis and methylation. One common genetic variation, the C677T polymorphism, leads to a substitution in the enzyme's structure (alanine to valine), significantly reducing its activity—especially in individuals with low folate intake (Frosst et al., 1995).

This reduced activity may impair DNA methylation and repair, contributing to genomic instability, which is a key factor in cancer development, including CLL.

### 6.1. Genetic and Metabolic Effects

The C677T and A1298C variants in the *MTHFR* gene reduce the enzymatic activity of methylenetetrahydrofolate reductase, thereby disrupting the folate cycle. This disruption leads to increased homocysteine levels, decreased DNA methylation, and impaired DNA repair. These metabolic and genetic alterations can increase cellular vulnerability to chromosomal instability for malignant transformation (Frost et al., 1995).

### 6.2. Cancer Risk

Several studies have linked MTHFR gene variants, particularly C677T and A1298C, with an increased risk of various cancers, including CLL. These mutations are thought to contribute to cancer susceptibility by impairing folate metabolism, reducing DNA methylation, and compromising genomic stability (Araszkiewicz et al., 2025). However, results vary across different populations and ethnic groups, suggesting the influence of additional genetic and environmental factors.

### 6.3. Role of MTHFR Polymorphisms in CLL

In CLL, MTHFR gene polymorphisms may play a significant role in disease susceptibility, treatment response, and drug metabolism. These genetic variations influence folate metabolism, which is crucial for DNA methylation and nucleotide synthesis, potentially affecting leukemogenesis and disease progression (Araszkiewicz et al., 2025).

MTHFR polymorphisms have been linked to treatment response, particularly to antifolate drugs such as methotrexate and fludarabine, which rely on folate pathways for their mechanism of action. Alterations in folate availability due to MTHFR variants may impact drug efficacy and toxicity, making genetic screening a potential tool for optimizing personalized treatment strategies (Skånland & Mato, 2021).

Additionally, these variants may affect drug metabolism, modify intracellular folate levels and influence the effectiveness of chemotherapy regimens. Individuals carrying the T allele may have an increased risk of hematologic malignancies, although further research is needed to confirm these associations (Aw et al., 2022).

### 6.4. Gene–Environment Interaction

The effect of MTHFR polymorphisms is likely influenced by dietary folate intake. Low folate levels combined with C677T mutations may cause DNA hypomethylation, increasing cancer risk (Skibola et al., 2002). Thus, nutrition and genetics together shape disease risk.

### 6.5. Neurological and Cardiovascular Effects of MTHFR

Variants in the *MTHFR* gene, particularly C677T, reduce enzyme activity and disrupt homocysteine metabolism. Elevated homocysteine is associated with increased risks of cardiovascular diseases, such as stroke and coronary artery disease, and has also been linked to cognitive decline and depression due to impaired methylation pathways (Van Der Put et al., 1995).

### 6.6. Fertility and Pregnancy

Fertility and pregnancy are not main concerns in CLL, but some genetic changes—especially in the MTHFR gene—might affect reproductive health. Variants like C677T and A1298C can disturb folate metabolism, causing high homocysteine levels, which are linked to negative reproductive effects in the general population.

In women, MTHFR mutations can disrupt folate metabolism, leading to problems like recurrent miscarriage, preeclampsia, and growth restric-

tion in the womb. Cao et al. (2013) found that the MTHFR C677T variant is linked to unexplained recurrent pregnancy loss, especially in East Asian women.

In men, MTHFR polymorphisms are linked to abnormal DNA methylation and sperm damage, which can lower fertility. Wu et al. (2010) found that increased methylation of the MTHFR gene in sperm is related to unexplained male infertility, indicating it may affect sperm production.

Therefore, assessing *MTHFR* status in reproductive-age CLL patients could offer additional insight into fertility risks and assist in counselling and management.

### 6.7. Clinical Relevance in CLL

Recent studies highlight that MTHFR gene polymorphisms may influence the progression, immune regulation, and treatment response in CLL. These polymorphisms can lead to epigenetic modifications, such as alterations in DNA methylation patterns, which may affect tumor suppressor gene expression and contribute to leukemogenesis (Kulis et al., 2012).

Recent evidence highlights that CLL cells contribute to immune evasion by directly inducing T-cell dysfunction. Specifically, CLL cells express Siglec-10 ligands such as CD24 and CD52, which interact with inhibitory receptors on T cells, leading to impaired immune responses. This immune dysregulation facilitates tumor survival and progression by suppressing effective anti-tumor immunity within the tumor microenvironment (van Bruggen et al., 2024).

What's more, MTHFR variants may also contribute to drug resistance, especially against targeted therapies such as BTK inhibitors. Epigenetic alterations can influence the efficacy of these therapies, posing challenges in treatment. Emerging research on non-covalent BTK inhibitors, such as pirtobrutinib, indicates potential in overcoming resistance associated with covalent BTK inhibitors (Wang et al., 2023).

Understanding these genetic and epigenetic influences is crucial for the development of biomarkers and precision medicine approaches, aiming to improve treatment strategies and outcomes for patients with CLL.

## 7. Future Perspectives and Clinical Trials

Recent advances in CLL research focus on shorter-duration therapies, overcoming drug resistance, and new immunotherapy options. These developments aim to improve long-term outcomes while reducing treatment burden.

### 7.1. Time-Limited Therapy

Fixed-duration treatment regimens, such as venetoclax combined with obinutuzumab or ibrutinib combined with venetoclax, have gained increasing attention in recent years. These time-limited approaches aim to:

➢Induce deep remissions, often characterized by undetectable minimal residual disease (uMRD)

➢Enable treatment discontinuation without compromising long-term disease control

Clinical trials have demonstrated that such strategies yield promising outcomes, particularly in the first-line treatment setting (Al-Sawaf et al., 2020). These regimens offer a significant shift from traditional continuous therapy by providing durable responses and improving patients' quality of life through defined treatment periods.

### 7.2. Next-Generation BTK Inhibitors

A key reference discussing next-generation BTK inhibitors pirtobrutinib targets resistance mutations like C481S in CLL (Naeem et al., 2023)

### 7.3. CAR T-Cell Therapy and Bispecific Antibodies

CAR T-cell therapy directed at CD19 has shown durable remissions in some patients with relapsed/refractory CLL. However, T-cell dysfunction in CLL limits its widespread success (Porter et al., 2015).

As an alternative, bispecific antibodies—such as mosunetuzumab—redirect the patient's own T cells to attack CLL cells by simultaneously binding CD3 (on T cells) and CD20 (on B cells). Early-phase trials suggest promising efficacy and manageable side effects (Budde et al., 2022).

### 7.4. Recent Advances in Immunotherapy for CLL

Recent developments in CLL immunotherapy focus on enhancing immune responses and overcoming tumor-induced immune suppression. Several investigational approaches are being explored to improve treatment efficacy:

Cancer vaccines aim to activate T-cell-mediated immunity against CLL-specific antigens, promoting an immune response capable of targeting malignant cells. These vaccines are designed to stimulate antigen-specific T-cell expansion, improving immune surveillance and tumor elimination (Hernandez & Malek, 2022).

Checkpoint inhibitors, such as anti-PD-1 and PD-L1 therapies, work to reverse immune exhaustion by restoring T-cell functionality. While their efficacy in CLL remains under exploring, they have shown promise in Richter transformation, a more aggressive form of the disease (Ding et al., 2017).

Microenvironment-targeting drugs focus on modulating the tumor microenvironment to enhance T-cell function and reduce immune evasion mechanisms. These therapies aim to reprogram immune cells within the tumor niche, improving the efficacy of immunotherapy (Chung et al., 2025).

Combination strategies integrating these approaches are currently undergoing clinical evaluation, with early trials suggesting that cancer vaccines, checkpoint inhibitors, and microenvironment-modifying therapies may significantly improve treatment responses in CLL.

**8. Epigenetic Alterations in CLL**

Epigenetic alterations play a significant role in the development and advancement of CLL, exerting their effects on gene expression without modifying the DNA sequence itself. Aberrant DNA methylation, particularly the hypermethylation of tumor suppressor genes such as DAPK1 and ZAP70, is frequently found in CLL and is related to aggressive disease features and poor prognosis (Kipps et al., 2022). Such epigenetic silencing inhibits apoptotic pathways and facilitates the survival of malignant B lymphocytes.

Therapy strategies targeting epigenetic regulators are under active investigation. Agents such as histone deacetylase (HDAC) inhibitors and DNA hypomethylating drugs have shown promise in reversing abnormal epigenetic signatures and restoring normal gene function (Zhang et al., 2025). These approaches may enhance responsiveness to conventional therapies and provide novel options for patients with relapsed or refractory CLL.

Furthermore, microRNAs (miRNAs), particularly miR-15a and miR-16-1, play a key role in CLL pathobiology by regulating the expression of anti-apoptotic genes, especially BCL-2. Deletions on chromosome 13q, which contains these miRNA loci, lead to their downregulation, contributing to impaired apoptosis, a hallmark of CLL development (Calin & Croce, 2006).

Ongoing research into epigenetic and miRNA-mediated mechanisms holds the potential to uncover novel biomarkers and targeted therapeutic strategies, ultimately improving clinical outcomes in CLL.

## 9. Real-World Data and Long-Term Outcomes

Clinical trial results are crucial, but **real-world evidence** provides additional insight into how treatments perform outside controlled study environments. Long-term observational studies show that:

➢BTK inhibitors and BCL-2 inhibitors maintain durable responses beyond 5 years

➢Undetectable MRD is associated with better long-term outcomes (Hyak et al., 2022)

Registries like the Connect CLL Registry and ERIC collect data on treatment effectiveness, safety, and patient adherence in daily clinical practice (Mato, 2020). These sources are especially useful for:

➢Elderly or frail patients

➢Individuals with other chronic illnesses

➢Populations underrepresented in trials

Such data help refine treatment strategies and shape healthcare policies.

## 10. Emerging Technologies and Artificial Intelligence in CLL

Recent advances in artificial intelligence (AI), machine learning (ML), and computational imaging have begun to transform the landscape of CLL research and clinical practice. These technologies improve pattern recognition and enable more precise, personalized medicine.

### 10.1. Machine Learning for Prognostic Modelling

Machine learning algorithms can analyse large-scale genomic and clinical datasets to identify complex patterns and interactions. Unlike traditional statistical methods, ML can uncover hidden relationships, such as combinations of mutations or flow cytometry profiles, that influence disease progression or treatment response. For example, unsupervised clustering of genomic mutations has been used to stratify patients more accurately (Kourou et al., 2015; Ko et al., 2020).

### 10.2. Digital Pathology and Radiomics in Oncology

Digital pathology tools, integrated with deep learning models, enable automated analysis of bone marrow and lymph node biopsies. These models classify cell morphology and detect subtle histological features predictive of aggressive disease. Similarly, radiomics, quantitative analysis

of imaging features extracted from CT and PET scans, has demonstrated potential in evaluating tumor burden and predicting treatment outcomes. Recent advancements in AI-driven radiomics have improved prognostic accuracy, aiding clinicians in personalized treatment planning (Peng et al., 2019).

### 10.3. AI in Personalized Treatment Planning

AI-powered decision support systems are increasingly being developed to assist in the individualized treatment of CLL. These models utilize deep learning and explainable artificial intelligence to analyse genetic expression profiles and predict the time to therapy. By integrating clinical and molecular data, AI systems can help identify high-risk patients and guide therapy decisions such as choosing between BTK inhibitors, BCL-2 inhibitors, or combination regimens (Morabito et al., 2023).

### 10.4. Integration into Real-World Data Platforms

Large registries and real-world data platforms are increasingly integrating AI tools to track treatment effectiveness, adverse events, and long-term outcomes. This integration allows for adaptive learning models that improve over time and better reflect clinical diversity (Kabadi et al., 2020).

## 11. Health-Related Quality of Life (HRQoL)

Health-related quality of life (HRQoL) is a key outcome in the treatment of CLL, particularly for patients with relapsed or refractory disease. Beyond survival, maintaining or improving patients' physical and emotional well-being has become a central focus of modern CLL therapy. The phase 3b VENICE II trial assessed the effects of venetoclax monotherapy on HRQoL and found clinically meaningful improvements, including a 9.3-point increase in global health status scores and notable gains in fatigue, insomnia, and role functioning subscales of the EORTC QLQ-C30 questionnaire (Cochrane et al., 2021).

Complementing these findings, the DEVOTE study, conducted in routine clinical settings across Canada, evaluated venetoclax-based therapy's impact on HRQoL. Patients reported rapid and sustained improvements in domains such as fatigue, physical functioning, and overall well-being starting from the end of the dose ramp-up phase through to the continuation phase of treatment (Aw et al., 2022). These studies collectively underscore the importance of incorporating patient-reported outcomes into therapeutic decision-making and highlight venetoclax as a treatment that not only controls disease effectively but also significantly enhances daily quality of life for CLL patients.

## 12. Pharmacoeconomic Considerations

Targeted therapies have significantly improved outcomes in chronic lymphocytic leukemia (CLL); however, their high costs raise sustainability concerns, particularly in lower-income countries. Economic evaluation tools like the incremental cost-effectiveness ratio (ICER) and quality-adjusted life years (QALY) are instrumental in assessing the value these treatments offer relative to their costs.

Fixed-duration venetoclax-based therapies, such as venetoclax plus obinutuzumab (VenO), have demonstrated cost advantages over continuous Bruton tyrosine kinase inhibitor (BTKi) treatments. For instance, a U.S.-based cost-effectiveness analysis found that VenO was more effective and less costly than several comparators, including ibrutinib and bendamustine plus rituximab, making it a dominant treatment option in certain scenarios (Chatterjee et al., 2021; Huntington et al., 2024).

Despite these advantages, access to VenO remains a challenge in resource-limited settings. A real-world study among U.S. Medicare beneficiaries indicated that while initial treatment costs for VenO and BTKi therapies were similar, VenO's fixed-duration approach led to significantly lower healthcare costs after the 12-month treatment period (Manzoor et al., 2024). These results highlight the importance of aligning clinical efficacy with economic sustainability, especially in healthcare environments where financial resources are limited.

## 13.Conclusion

CLL is a biologically and clinically heterogeneous disease that has undergone a profound transformation over recent decades. Improves in molecular biology, particularly the identification of recurrent genetic mutations and variants such as *MTHFR C677T*, have significantly enhanced our understanding of disease pathogenesis and treatment responses.

Targeted therapies, including BTK inhibitors and BCL-2 inhibitors, have largely supplanted traditional chemoimmunotherapy in many clinical settings. These agents offer improved survival outcomes and enhanced quality of life for patients. Contemporary management strategies increasingly emphasize time-limited treatment, overcoming drug resistance, and leveraging immunotherapeutic tools such as CAR T-cell therapy, bispecific antibodies, and immune checkpoint inhibitors.

In parallel, emerging technologies—including artificial intelligence, epigenetic profiling, and the analysis of real-world data—are driving the evolution of personalized medicine in CLL. These innovations allow for

more precise risk stratification, therapy selection, and monitoring of treatment response.

Despite substantial progress, several critical challenges remain. Future research should aim to:

➢Elucidate the clinical significance of various genetic polymorphisms.

➢Improve the cost-effectiveness and scalability of novel therapies.

➢Ensure equitable access to innovative treatments on a global scale.

In conclusion, CLL is now managed as a genetically informed, precision-guided malignancy, with ongoing research expected to further refine therapeutic approaches and improve patient outcomes worldwide.

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# Chapter 3

# INNOVATIVE NANOENZYME APPROACHES IN CANCER THERAPY

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## Introduction

Enzymes are highly effective tools for use in vitro, as they catalyse biotransformations with exceptional selectivity and specificity. They are also highly biocompatible and environmentally benign. In this respect, they offer an excellent green alternative to chemical catalysts. Technologies such as enzyme immobilisation and enzyme-driven evolution have made the use of enzymes in vivo more feasible by enabling their repeated use and storage (Basso and Serban 2019). Thanks to recent advances, enzymes are now widely used in the production of active pharmaceutical ingredients (APIs), health supplements, agrochemicals and biofuels, as well as in food processing, environmental monitoring, and the diagnosis and treatment of various diseases (Datta *et al*., 2020).

Since the feasibility of using enzymes in therapies was discovered, various strategies have been developed rapidly to catalyse critical biochemical reactions with a healing effect in the body. Initially, these therapies were used to treat rare genetic diseases caused by a deficiency or dysfunction of an important metabolic enzyme. While these therapies provided a temporary solution, they required lifelong intake of the missing or defective enzyme. However, several clinical trials have demonstrated the high efficacy and safety of enzymes in treating these rare inherited diseases (Brady, 1966).

Enzymes have since been investigated for their effectiveness in treating infectious diseases (Heselpoth *et al*., 2018) cancer (Dinndorf *et al*., 2007; Hito and Chandra, 2015), wound healing (Kurahashi and Fujii, 2015; Barker *et al*., 2017), inflammatory diseases, neurodegenerative diseases such as Alzheimer's (Nalivaeva and Turner 2019; Sikanyika *et al*., 2019), and gene-editing therapies(Villa *et al*., 2016; Torres-Herrero *et al*., 2024). However, the technology has not yet been established, and its applications have not become widespread (Dinndorf *et al*., 2007).

Enzymes with therapeutic properties play a role in treatment in two ways. The first is the reaction of the enzyme with its substrate to produce a therapeutic agent that corrects abnormal physiological or metabolic pathways. This is called enzyme replacement therapy (ERT). The second is the treatment method in which the enzyme indirectly activates prodrugs, this method is called enzyme prodrug therapy (EPT). Clinical effects can be obtained by using enzyme dynamics therapies (EDT) or enzyme starvation therapies (EST) by considering host substrate molecules in the indirect use of enzyme in treatments. Regardless of the route taken, treatment with enzymes can offer advantages over conventional treatment. The high affinity of enzymes, substrate selectivity, the ability

to perform multiple reactions with a single enzyme and their degradability at the end of the process provide advantages in treatments. In addition to these features, their short life span in the body, inability to pass through barriers such as cell membrane or blood-brain barrier, relatively costly production, side effects due to immunogenicity or OFFtarget accumulation are seen as disadvantages, and research are being developed to overcome these problems (Datta *et al*., 2020; Torres-Herrero *et al*., 2024).

To overcome these problems, nanomaterial and protein engineering studies have been integrated. Protein engineers can optimise the stability of enzymes, their affinity for natural or non-natural substrates and increase reaction efficiency (Villa *et al*., 2016; Graham *et al*., 2018; He *et al*., 2018). In addition, with newly developed techniques such as de novo design, directed evolution and rational design, the affinity of enzymes can be increased, enzymes can work more stable under physiological conditions and various side effects can be eliminated.

The term 'nanoenzyme' was first coined in 2004 when gold nanoparticles were found to be capable of transphosphorylation due to the spontaneous binding of thiols to their surface (Manea *et al*., 2004). Subsequent studies have demonstrated that inorganic nanomaterials can imitate the function of enzymes (Wu *et al*., 2019). The term 'nanoenzymes' was coined in 2013 to describe nanomaterials with enzyme-like properties (Wei and Wang, 2013). Although nanoenzymes follow the same metabolic pathway as their counterpart enzymes, it is sufficient that they form the same product using the same substrate. However, they have several advantages compared to their counterparts (Huang *et al*., 2019). In addition to their catalysis ability, their bioconjugation capabilities and self-assembly capabilities are the advantages of their nano size. Nanoenzymes with magnetic properties provide great advantages in purification and in vivo MR (magnetic resonance) imaging. Some nanoenzymes have surface plasmon properties and their photothermal properties provide advantages in cancer treatment. In addition to their enzyme-like catalysis capabilities, their multifunctionality makes a difference in biomedical imaging, treatment and diagnosis, and bioanalysis. Therefore, many nano equivalents of ligases, hydrolases and oxidoreductases have been produced [Wei and Wang, 2013; Wu *et al*., 2018; Huang et al., 2019; Wu *et al*., 2019].

**Nanoenzym**

Structures called nanoenzyms are materials obtained from nanomaterials (1-100 nm) and could mimic enzyme-like catalytic reactions. They can catalyse enzyme reactions under in-vivo conditions. For example, nanoparticles made with $Fe_3O_4$ can mimic peroxidases (POD) (Wellen-

stein *et al.,* 2019). Oxidases (OXD), catalases (CAT) and superoxide dismutases (SOD) can also be mimicked like PODs [Table 1] (Gomaa 2022).

**Table 1**. *Some nanoenzyme structures and their activities (Gomaa 2022).*

| **Nanoenzym** | **Enzyme-like activity** | | | |
|---|---|---|---|---|
| | **POD** | **CAT** | **SOD** | **OXD** |
| His-AuNCs | x | | | |
| $CuInS_2$ NC | x | | | |
| $CeO_2$ NPs | x | x | x | |
| $Fe_2O_3$ NPs | x | | | |
| $ZnFe_2O_4$/ZnO NC | x | | | |
| CoFe LDHs | x | | | |
| $MnFe_2O_4$ NC | | | | x |
| $Fe_3O_4$ NPs | x | x | | |
| $CoFe_2O_4$ NPs | | | | x |
| $CePO_4$: Tb, Gd NPs | x | | | |
| $Fe_3O_4$ | x | x | | |
| Au/CuS NC | x | | | |
| $Mn_3O_4$ octahedrons | | | | x |
| $VO_2$ nanoplates | x | | | |
| MnSe NPs | x | | | |
| CuO nanostructure | x | | | |
| $Fe_3O_4$ NPs | x | x | | |
| $FePO_4$ microflowers | | | x | |
| $Co_3O_4$ NPs | x | x | x | x |
| $CeO_2$ NPs | x | x | x | x |
| $V_2O_5$ nanowires | x | | | |

Enzyme-like nanoparticles based on gold, carbon, graphene oxide, iron oxide and $CeO_2$ are being applied and investigated. As a result of research carried out in recent years, it is thought that 550 different nanoenzymes have been discovered, with this number increasing day by day.

Nanoenzyms can regulate the redox levels of cells through enzymatic reactions (Wei and Wang, 2013). Reactive oxygen species (ROS) are harmful by-products of oxygen metabolism. Increased amount of ROS in cells causes oxidative stress and disrupts redox homeostasis. Oxidative stress occurs as a result of inadequate detoxification of ROS by the body (Wu *et al*., 2019). Nanoenzyms can maintain intracellular ROS balance with catalase and superoxide dismutase activity (Xia *et al.,* 2020). At the same time, ROS production may increase with peroxidase and oxidase activities of nanosyms, which may trigger apoptosis in cancer cells.

Nanoenzymes are widely used in cancer treatment and a variety of other medical applications, as well as in environmental and industrial fields. This is because they can be produced using simple, inexpensive materials and offer high stability and catalytic efficiency (Zhang *et al*., 2019).

Nanoenzymes can be produced with a variety of functions, including oxidoreductase, hydrolase, ligase, translocase, isomerase, transferase and lyase activities. As can be seen in Table 1, the majority of nanoenzymes exhibit OXD, POD, CAT and SOD-like activities. A small portion of the nanosyms (Figure 1) with these activities show their activities together with hydrolases (Chong *et al*., 2021).

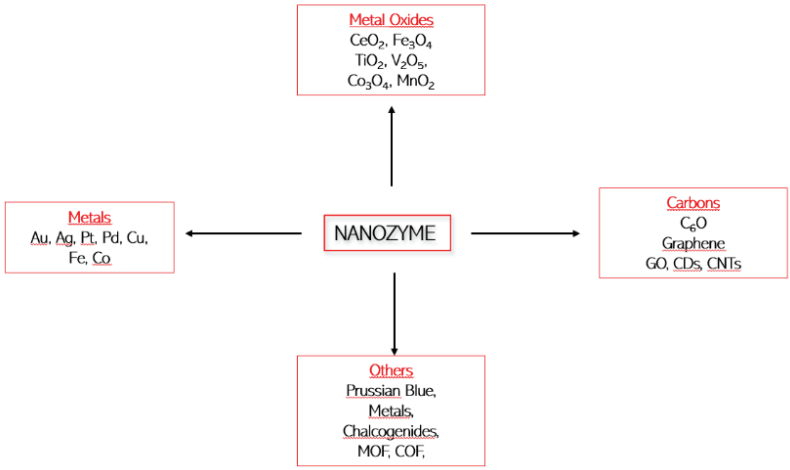


**Figure 1.** *The classification of nanoenzymes is important for cancer treatment and diagnosis (Shahid et al., 2024).*

**Nanoenzymes and Cancer Therapy**

As we have already mentioned, conventional treatments have a poor prognosis and cause side effects. Thanks to their enhanced permeability and retention (EPR) properties, nanoenzymes can target peptides and reach cancerous cells (Li *et al*., 2019a; Li *et al*., 2019b). There are two ways in which nanoenzymes can kill cancerous cells: firstly, they can increase the amount of ROS accumulated in cells using their oxidase and peroxidase capabilities. This directly attacks the tumour cells. Secondly, they can reduce the tumour's microenvironmental hypoxia by using SOD and CAT activities. This method should be supported by chemotherapy and radiotherapy. The second way is indirect killing of cancer cells (Ding *et al*., 2020).

**Breast and Ovarian Cancer**

Cancer is one of the diseases that cause the most deaths and risk factors are increasing due to people's lifestyle, which will lead to an increase in cancer cases (Tran *et al.,* 2022). Cancer incidence rate is higher in developed countries (Momenimovahed and Salehiniya, 2019). The most common types of cancer are lung and breast cancer, respectively. Breast cancer ranks 5th among the causes of human death (Giaquinto *et al*., 2022). The rate of breast cancer is expected to increase by 30% in Western countries due to women's use of menopausal hormonal drugs and changes in reproductive regulations (Lee et al., 2019). However, since the beginning of the 20th century, it has been observed that this rate has remained constant and relatively decreased. It is thought that awareness activities increase sensitivity to risk factors. In less developed countries, the same decrease has not been observed due to the problems experienced in screening (Fahad Ulah, 2019).

Breast cancer is the most common malignancy in women and is heterogeneous. Considering these features, many treatments have been developed in the last 15 years, focusing on biologically directed therapies to reduce the side effects of these treatments (Ezzati et al., 2020). In cases where the tumour is in the breast or in the lymph nodes, which is called early stage, it is seen that the possibility of treatment is very high. Developed multimodal therapies have increased this possibility. Metastasised cancer patients cannot be treated with current therapies (Fachal et al., 2020). Advanced breast cancer patients can be prolonged by controlling the patient's symptoms with low treatment-associated side effects and by improving the patient's lifestyle (Dorling *et al*., 2021).

Ovarian cancer is accepted as the most common gynaecological tumour in the literatüre (Silvestris *et al*., 2019). Ovarian cancer, which has

a high mortality rate, is considered 3 times more dangerous than breast cancer. But it is not as common as breast cancer. While genetic, lifestyle and environmental factors generally cause ovarian cancer, the risk can be reduced by pregnancy, breastfeeding and the use of some hormonal drugs (Momenimovahed *et al*., 2019).

Ovarian cancer treatment options include advanced surgical interventions, chemotherapy and radiotherapy. Patients are first treated with taxane or platyl-based chemotherapy and then the tumour is removed as much as possible by surgical intervention. There is usually a high rate of improvement after these treatments, but relapse within 2 years is very likely. Long-term treatment leads to the development of drug resistance. Personalised treatments are thought to be a hope for ovarian cancer treatment. Treatments can be developed by using atomic properties common to the tissues in which tumours occur. (Delie *et al*., 2012).

Nanoenzymes can be used in various ways in the diagnosis and treatment of ovarian cancer. They can be used as biomarkers in cancer diagnosis, multiplex detection and non-invasive imaging. They can also be used in breast cancer treatment with strategies such as drug delivery to target tissue photothermal therapy (PTT), immunotherapy and radiotherapy enhancement. (Golchin *et al*., 2017; Kurnit *et al*., 2021).

Nanoparticles have been found to trigger autophagy, the body's ability to produce healthy cells and clean damaged cells (Golchin *et al*., 2017).

Nanoenzymes have the ability to kill breast cancer cells by increasing the amount of intracellular ROS through their oxidase and peroxidase activities. The large amounts of $H_2O_2$ that accumulate around cancer cells are converted into stotoxic free radicals by peroxidase catalysis. Regulating the amount of ROS was achieved by producing nitrogen-doped and porous nanosims that do this (Fan *et al.,* 2018). Carbon nitrogen nanosims can increase the amount of ROS by utilizing oxygen peroxide and hydrogen in acidic environment (An *et al.,* 2013).

Carbon nitrogen-containing ferritin nanoparticles produce ROS that directly target cancer cells, especially in lysosomes, while catalysing $H_2O_2$ and hydrogen. Animal experiments have also proven the effect of these nanosims in controlling tumour growth (Fan *et al*., 2018). In addition, B7-H4 combination therapy has been shown to suppress the progression of breast cancer. The production of high amounts of ROS by ascorbic acid also alters the redox balance in cancer cells. However, there are studies proving that the efficacy of the doses of ascorbic acid suitable for the body in intracellular experiments is not sufficient (An *et al*., 2013).

The oxidative properties of cerium oxide-based nanoparticles (CNPs) at acidic pH trigger cell death by causing an increase in the amount of ROS. The advantage of CNPs over other nanosims that are effective in cancer cells is that they are renewable and reduce ROS levels in healthy cells and pro-oxidants in cancer cells (Alili *et al*., 2011). CNPs (also known as nanoceria) prevent cancer cells from metastasising by inhibiting the growth of myofibroblasts (Das *et al.,* 2017).

Nanocerias reduce cell proliferation in ovarian cancer cells in vivo and in vitro and decrease cell motility and aggressiveness. Folate-conjugated (FA-$CeO_2$ at 24.3+) nanoceria has been proven to decrease ovarian cancer cell expansion and increase ROS age (Giri *et al*., 2013; Das *et al.,* 2017).

**Skin Cancer**

Nanoenzyms showing catalase activity are preferred in skin cancer cases. A treatment based on the breakdown of hydrogen peroxide ($H_2O_2$) in skin wounds into oxygen and water is aimed (Sen 2019). High ROS accumulation and oxidative concentration are observed in chronic wounds. Slow revascularisation of wounds prevents access to the oxygen needed for oxygenation. Nanoenzyms with CAT activity can promote oxygen production in the wound by reducing or eliminating ROS formation in wounds. This will lead to wound healing.

The nanoenzymes used in skin cancer treatment that exhibit CAT activity are usually made of molybdenum disulfide ($MoS_2$), manganese dioxide ($MnO_2$) or manganese cobalt oxide (MnCoO) (Han *et al*., 2023). In addition to the CAT activity of $MoS_2$, its high biocompatibility (Ren *et al*., 2020) and its antioxidant, anti-inflammatory and antimicrobial properties were advantageous in the studies. The hydrogel formed as a result of the reaction scavenged ROS and reactive nitrogen species (RNS), reduced inflammation, and destroyed bacteria with photothermal therapy (PTT). It showed synergistic effect in PTT applications together with dressing in advanced wound care (Li *et al*., 2022).

**Lung Cancer**

The most important reason for the high mortality rate of patients with non-small cell lung cancer (NSCLC) is that it is not diagnosed at an early stage. Early detection is very important for lung cancer. NSCLC screening is performed by low-dose computed tomography (LDCT), but short interval scans for early diagnosis cause cumulative radiation exposure in patients (Detterbeck *et al*., 2013). The liquid biopsy method involves extracting and analysing tumour DNA from the bloodstream and exosomes from the patient's body fluids. The fact that it is non-invasive, reproduc-

ible and generally applicable to all patients makes this method advantageous (Bettegowda et al., 2014; Santarpia et al., 2018).

Exosomes are small vesicles secreted by both healthy and tumour cells. They are biomarkers that play an important role in the progression and diagnosis of NSCLC (Yáñez-Mó *et al*., 2015). Studies have shown that NSCLC patients have very high levels of exosomes in their blood compared to healthy people (Rabinowits *et al*., 2009).

In recent years, the production of immunoreaction-based biosensors has gained momentum due to their rapid results and miniaturisation capabilities (Wei *et al*., 2023). Aptasensors are of most interest, aptamers are used in the measurement. They are advantageous with their easy use, fast results and low cost (Tang *et al*., 2022). Aptasensors have been shown to be easily improved by various studies. Although the catalytic ability of natural enzymes is very high, they are easily affected by environmental conditions, leading researchers to alternatives (Boyarski et al., 2023).

Iridium oxide nanoparticles ($IrO_2$NP) are nanoscale oxide compounds produced from the element iridium. They are highly preferred in electrochemical reactions as nanoenzyms in oxygen reduction reactions (Gu *et al*., 2019). They have high stability and continue to work without degradation in corrosive and oxidative environments (Zhao *et al*., 2021; Quinson 2022). In addition to all these, their high peroxidase-like activity is preferred in immunological analyses (Joshi *et al*., 2020; Xu *et al*., 2024).

**Liver Cancer**

Hepatocellular carcinoma (HCC) is a type of liver cancer. It is insidious in the early stages, characterised by a high mortality rate and poor prognosis. It is the fourth most common type of cancer and the second biggest cancer-related killer. The five-year survival rate is quite low, at 10% (Sung *et al*., 2021). These characteristics make HCC a subject of research. The traditional treatment method consists of surgical intervention, radiotherapy, and chemotherapy (Anwanwan et al., 2020). Since the likelihood of recurrence is high after surgery, radiotherapy and chemotherapy should be used as supportive treatments. However, since these methods also damage healthy cells in addition to cancer cells, it is essential to seek alternative treatments (Hefnawy *et al*., 2024).

Methods such as photodynamic therapy (Luo and Gao 2023) chemical dynamic therapy (Jia *et al*., 2022), and photothermal therapy (Wang *et al*., 2022; Li *et al*., 2024) are alternatives. Anoikis is a condition where the connection between epithelial cells and the extracellular matrix is disrupted, leading to programmed cell death. This property can be used to

inhibit the proliferation and survival of cancer cells (Taddei *et al*., 2012; Paoli *et al*., 2013; Zhao *et al*., 2023). This treatment has been validated in endometrial (Chen *et al*., 2021), lung (Jin *et al*., 2018), prostate (Liang *et al*., 2023), esophageal (Chen *et al*., 2018) and stomach (Ye et al., 2020) cancers.

Epithelial-mesenchymal transition (EMT) promotes metastasis and is effective in tumor growth and spread. It is also an important process in acquiring anoikis resistance (AR). The acquisition of AR through EMT is an important indicator of tumor cell metastasis and disease recurrence [Liotta, and Kohn 2004; Simpson *et al*., 2008). A nanosystem design capable of inhibiting the AR ability of HCG cells will prevent disease recurrence and metastasis after surgery. In a study conducted for this purpose, MSN-Ce@SP/PEG nanoenzymes were developed, and it was reported that they disrupted the EMT ability of HCC cells and successfully prevented metastasis and disease recurrence. With this nanoenzyme, we can see how effective nanoenzymes can be in the treatment of tumors associated with AR (Wang *et al.,* 2025).

In addition, heterogeneous glucose levels in HCC tumor microenvironments also pose challenges during treatment. To address this issue, organosilicate nanoparticles with hollow mesopores have been designed. Glucose oxidase (GOx) and the $Fe^{2+}/Fe^{3+}$ redox pair are co-loaded. Glutathione (GSH) is concealed in the tumor cell membrane for high selectivity. This complex nanoenzyme system is referred to as M@GOx/Fe-HMON.

In regions rich in glucose, the designed nanoenzyme produces ROS with GOx-peroxidase (POD) as a result of the Fenton reaction. Ferroptois occurs as a result of increased oxidative stress and depletion of GSH in the environment. In glucose-depleted regions, the designed nanoenzyme further reduces glucose levels by utilizing nicotinamide adenine dinucleotide phosphate (NADPH), disrupting the cysteine metabolic pathway and promoting disulfideptosis, a form of programmed cell death. The result is actin cytoskeletal collapse. This nanoenzyme induces disulfideptosis or ferroptosis depending on the amount of glucose around the tumor. As a result, redox homeostasis is disrupted, and tumor growth is inhibited. Two metabolisms are activated with a single nanoenzyme (Zhou *et al*., 2025).

## Conclusions

All the nanoenzyme treatments mentioned in this section were studied within the last two years, considering when the book was written. Clearly, new advantages will be added to the numerous existing benefits of nanoenzymes. As cancer cell characteristics, biochemical environments, and modes of spread are investigated further, new nanoenzyme therapies are anticipated to emerge. Based on the available data, nanoenzymes show promise as a new approach to cancer treatment.

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# Chapter 4

# FUTURE THERAPEUTIC STRATEGIES FOR METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE (MAFLD):EMERGING TARGETS AND PRECISION MEDICINE APPROACHES

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## Introduction

Metabolic dysfunction-associated fatty liver disease (MAFLD), previously referred to as nonalcoholic fatty liver disease (NAFLD), is characterized by hepatic steatosis in conjunction with metabolic risk factors such as overweight/obesity, type 2 diabetes mellitus (T2DM), or at least two features of metabolic dysfunction(1). The global prevalence of MAFLD is estimated at 25–30 %, with up to 80 % prevalence among obese individuals and a significant share of those with T2DM . While many patients remain in the benign "simple steatosis" stage, approximately 20–30 % progress to metabolic dysfunction-associated steatohepatitis (MASH), which carries increased risks of fibrosis, cirrhosis, hepatocellular carcinoma, and cardiovascular morbidity(2)(Figure 1)(7). Until recently, the cornerstone of MAFLD management remained intensive lifestyle modification—targeting 7–10 % weight loss through low-calorie, Mediterranean or low-carbohydrate diets combined with moderate-to-vigorous physical activity—in order to reduce hepatic steatosis and improve histologic features(3). Pharmacologic strategies including pioglitazone, vitamin E (in non-diabetic patients with fibrosis), SGLT-2 inhibitors, GLP-1 agonists, and statins were recommended off-label based on cardiovascular or metabolic evidence(4). However, the recent FDA approval of resmetirom (Rezdiffra™), a thyroid hormone receptor-β agonist, marks a transformative step, validated by a phase III NASH trial showing MASH resolution and fibrosis regression at 12 months(5)

Updated 2024 clinical practice guidelines—EASL-EASD-EASO and APASL—emphasize early identification of fibrosis using transient elastography and non-invasive biomarkers, stratified case-finding algorithms, and a personalized multipronged treatment model incorporating both lifestyle and emerging pharmacotherapies(6) In advanced stages, mono- and combination therapies targeting metabolic, inflammatory, and fibrotic pathways—such as FXR agonists, PPAR agonists, CCR2/5 antagonists, and novel dual/triple receptor co-agonists—are under intensive investigation(6,7)

This article provides a comprehensive overview of these recent guideline updates, evaluates current therapeutic options across all disease stages, and highlights ongoing clinical trials aimed at delivering next-generation therapies. By integrating current evidence with evolving pharmacological advances, we propose an optimized, stage-specific treatment algorithm for MAFLD/MASH aimed at improving patient-centred outcomes and reducing liver-related morbidity.

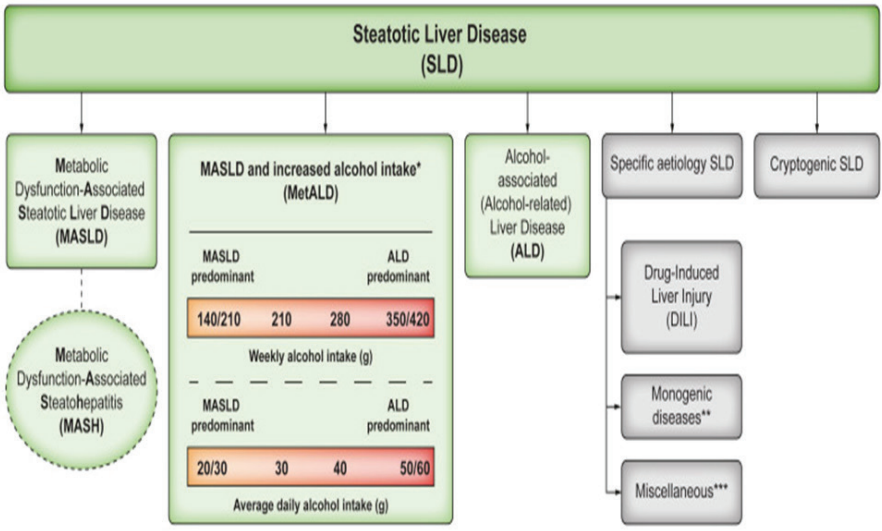


**Figure. 1**. *Steatotic liver disease (SLD) subclassification (7)*

## Pathogenesis

MAFLD is characterized by hepatic steatosis in conjunction with metabolic disturbances, including insulin resistance (IR), obesity, or type 2 diabetes mellitus (T2DM). The pathogenesis is multifactorial, involving lipotoxicity, mitochondrial dysfunction, inflammation, gut dysbiosis, and genetic predisposition.

**Table 1.** *The Pathogenesis od MAFLD*

| Pathogenic Axis | Mechanism | Therapeutic Implications |
|---|---|---|
| Energy & lipid imbalance | Insulin resistance → lipid overload | Lifestyle, ACC/FAS inhibitors |
| Lipotoxicity & organelle stress | Lipid intermediates → ROS, ER stress | Antioxidants, ER stress modulators |
| Ferroptosis | Iron + peroxidation → cell death | Ferroptosis inhibitors |
| Immune activation | DAMPs → Kupffer cell / macrophage activation | NLRP3, TLR4 antagonists |
| Gut–liver mediation | LPS, SCFAs, bile acids → hepatic signaling | Microbiome modulators |
| Programmed cell death | Necroptosis, pyroptosis | RIPK/MLKL, gasdermin inhibitors |
| Fibrosis | HSC activation, ECM deposition | TGF-β blockers, anti-fibrotics |
| Genetic/epigenetic modifiers | SNPs, PTMs, DNA methylation | Precision medicine, epigenetic modulation |

### A. Insulin Resistance and Dysregulated Lipid Metabolism

Metabolic dysfunction in MASLD is primarily driven by peripheral insulin resistance (IR), which leads to compensatory hyperinsulinemia. This hyperinsulinemic state activates lipogenic transcription factors such as sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate-responsive element-binding protein (ChREBP), both of which upregulate de novo lipogenesis (DNL) [1]. In addition, increased adipose tissue lipolysis and dietary fat intake elevate hepatic free fatty acid (FFA) flux, enhancing steatosis (8).

Genetic polymorphisms such as PNPLA3-I148M, TM6SF2, and MBOAT7 impair lipid droplet metabolism and VLDL secretion, worsening lipid accumulation and hepatocyte stress (9,10).

### B. Lipotoxicity and Oxidative Stress

Excess FFAs are oxidized in mitochondria, producing reactive oxygen species (ROS) and contributing to mitochondrial dysfunction. ROS-mediated damage compromises mitochondrial integrity and cellular homeostasis(11). Endoplasmic reticulum (ER) stress, coupled with JNK activation, promotes hepatocyte apoptosis and inflammatory responses(12).

Ceramides and other lipotoxic metabolites have been implicated in activating Kupffer cells and promoting fibrogenesis, marking a key transition point from steatosis to steatohepatitis(13).

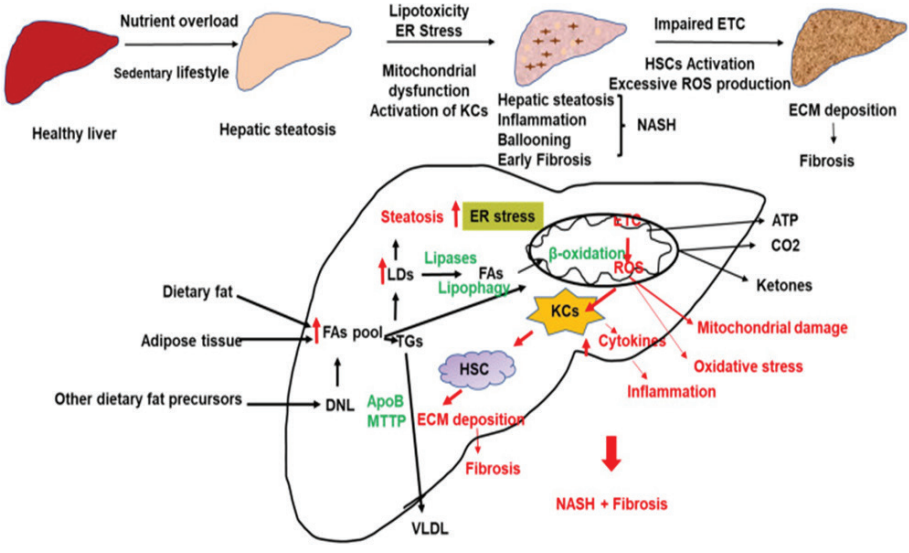


**Figure 2.** *Influence of current and emerging therapies on MAFLD (29)*

### C. Inflammation and Fibrosis

The hepatocellular injury characteristic of MASLD results in the release of damage-associated molecular patterns (DAMPs) from necrotic hepatocytes, which engage pattern recognition receptors on resident Kupffer cells (14,15). This interaction triggers NF-κB-mediated transcriptional activation, culminating in the robust secretion of proinflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and transforming growth factor-β (TGF-β) (14,15).

The resultant cytokine milieu establishes a chemotactic gradient that facilitates the recruitment of circulating inflammatory monocytes through the CCL2/CCR2 chemokine axis, a process mediated by vascular cell adhesion molecule-1 (VCAM-1) dependent endothelial transmigration(15). This cellular infiltration perpetuates a self-amplifying cycle of hepatic inflammation through additional cytokine production and reactive oxygen species generation.

The inflammatory microenvironment subsequently induces phenotypic activation of quiescent hepatic stellate cells (HSCs), which undergo transdifferentiation into proliferative, contractile myofibroblasts (16). This transformation is principally driven by TGF-β/Smad signaling, with concomitant upregulation of α-smooth muscle actin (α-SMA) and initiation of extracellular matrix deposition (16,17). The fibrogenic cascade is further amplified by platelet-derived growth factor (PDGF)-mediated HSC proliferation and tissue nhibitör of metalloproteinases (TIMP)-mediated inhibition of matrix degradation.

### D. Gut-Liver Axis and Microbiota Dysbiosis

The gut-liver axis constitutes a critical bidirectional communication system that plays a fundamental role in the pathogenesis of metabolic dysfunction-associated steatotic liver disease (MASLD). Emerging evidence demonstrates that gut microbial dysbiosis leads to intestinal barrier dysfunction characterized by tight junction protein disruption (e.g., occludin, claudin-1, and zonulin-1 downregulation), resulting in increased intestinal permeability (18,19).

This “leaky gut” phenomenon facilitates the translocation of pathogen-associated molecular patterns (PAMPs), particularly lipopolysaccharide (LPS), into the portal circulation. LPS activates hepatic TLR4 signaling on Kupffer cells and hepatocytes, triggering downstream NF-κB and JNK inflammatory pathways that promote the release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and drive hepatic insulin resistance (20).

**D.1 Therapeutic Perspectives:**

Current investigational approaches targeting the gut-liver axis include:

•Microbiota modulation: Probiotics (e.g., Akkermansia muciniphila), prebiotics, and fecal microbiota transplantation

•Barrier enhancement: Zonulin inhibitors (larazotide) and tight junction stabilizers

•Receptor-targeted therapies: FXR agonists (obeticholic acid), TGR5 agonists, and TLR4 antagonists (21).

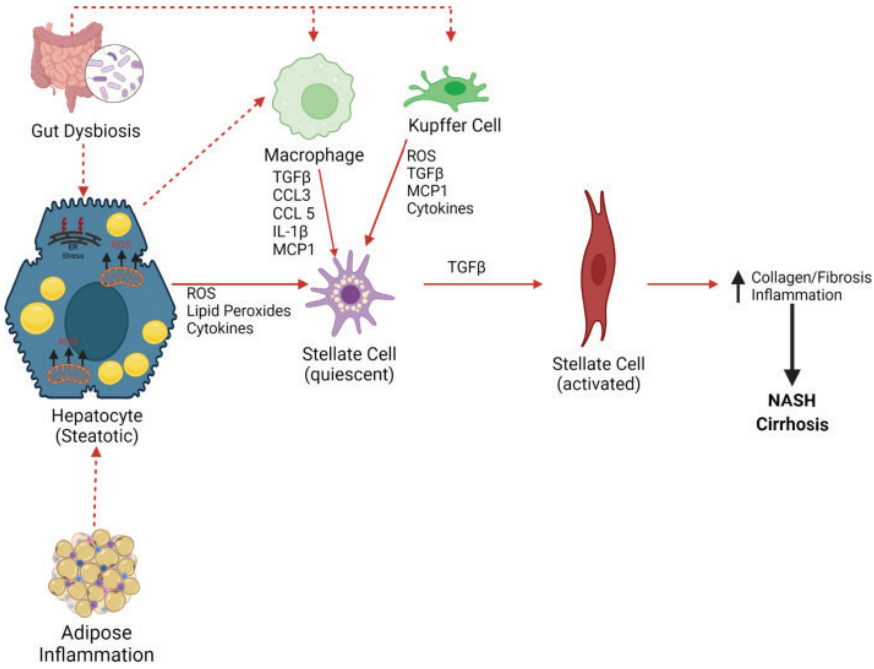


**Figure 3.** *Mechanism of hepatic stellate cell activation (29)*

**E. Programmed Cell Death Pathways**

Recent studies reveal that necroptosis (via RIPK1-RIPK3-MLKL) and pyroptosis (via gasdermin D) contribute to hepatocyte death in MASLD, amplifying sterile inflammation and accelerating disease progression.

Emerging evidence implicates regulated cell death (RCD) pathways, particularly necroptosis and pyroptosis, as critical drivers of hepatocyte injury in metabolic dysfunction-associated steatotic liver disease

(MASLD). These processes perpetuate sterile inflammation and accelerate disease progression through distinct molecular mechanisms:

**E1. Necroptosis in MASLD**

Signaling Cascade: Activated via RIPK1-RIPK3-MLKL axis under conditions of metabolic stress (e.g., lipotoxicity, oxidative stress) [5]. Phosphorylated MLKL forms pores in plasma membranes, causing cell rupture and release of damage-associated molecular patterns (DAMPs) (22).

Consequences:

•Amplifies Kupffer cell activation and TNF-α/IL-1β production (22).

•Correlates with fibrosis stage in clinical cohorts (14,22).

**E2. Pyroptosis in MASLD**

Molecular Triggers:

•NLRP3 inflammasome activation cleaves gasdermin D (GSDMD), generating membrane pores(22).

•Driven by mitochondrial ROS and ER stress in hepatocytes (23).

Downstream Effects:

•Releases pro-inflammatory cytokines (IL-18, IL-1β) and cellular contents.

•Promotes stellate cell activation and collagen deposition (14,23).

**E3. Therapeutic Implications**

•Necroptosis inhibitors: Targeting RIPK1 (e.g., necrostatin-1) reduced steatohepatitis in preclinical models(22).

•Pyroptosis blockade: Disulfiram (GSDMD inhibitor) attenuated fibrosis in murine MASLD (22,23).

•Combined approaches: Dual inhibition of RCD pathways may synergize with metabolic therapies (e.g., FXR agonists) (23).

**F. Genetic and Epigenetic Modifiers**

Genetic variants such as PNPLA3, TM6SF2, and MBOAT7 increase susceptibility to MASLD and its progression (24,25). Additionally, epigenetic changes like DNA methylation, histone acetylation, and non-coding RNAs influence lipid metabolism and fibrogenesis, as demonstrated

in both animal and human models (25).

**G. Post-Translational Modifications (PTMs)**

PTMs such as phosphorylation, ubiquitylation, acetylation, and glycosylation regulate proteins involved in lipid metabolism, inflammation, and fibrosis. Advanced proteomic analyses (e.g., mass spectrometry) indicate PTMs as potential therapeutic target(26).

A.Regulation of Hepatic Lipid Metabolism:

•Phosphorylation: AMPK phosphorylation at Thr172 inhibits lipogenesis while activating β-oxidation (7)

•Ubiquitylation: Proteasomal degradation of SREBP-1c reduces hepatic lipid accumulation (8)

•Acetylation: PGC-1α acetylation suppresses mitochondrial biogenesis (25)

B.Modulation of Inflammatory Responses:

•N-glycosylation: TLR4 glycosylation enhances LPS-mediated signaling (26)

•SUMOylation: Regulates nuclear translocation of NF-κB (26)

C.Fibrosis Progression:

•Lysyl oxidation: Catalyzes collagen cross-linking in ECM remodeling

•Palmitoylation: Modulates HSC activation and contractility (27)

**Table 2.** *Clinically Relevant PTM Targets (27)*

| PTM Type | Target Protein | Pathogenic Effect | Therapeutic Approach |
|---|---|---|---|
| O-GlcNAcylation | FOXO1 | Promotes insulin resistance | OGT inhibitors |
| Acetylation | STAT3 | Enhances pro-inflammatory signaling | HDAC inhibitors |
| Ubiquitylation | ACC1 | Regulates lipogenic activity | PROTAC molecules |

**Emerging Pharmacotherapies**

**A. Metabolic Pathway-Targeted Therapies(Figure 4)**

1. Thyroid Hormone Receptor-β (THR-β) Agonists: Hepatic THR-β activation enhances mitochondrial β-oxidation while suppressing de novo lipogenesis (28)

•Resmetirom: First-in-class selective agonist demonstrating: ≥30% relative reduction in hepatic fat fraction (28). Improved NASH resolution without worsening fibrosis ($p<0.001$ vs placebo)

Future Directions: Potential synergy with GLP-1 receptor agonists in ongoing combination trials

2. Fibroblast Growth Factor 21 (FGF21) Analogs

•Efruxifermin: Phase 2b data show 65-70% relative reduction in liver fat content. Significant improvement in fibrosis markers (≥1-stage improvement in 41% patients)(30)

•Pegbelfermin: Shows promise in reducing liver fat and inflammation.

3. Glucagon-Like Peptide-1 (GLP-1) and Dual Agonists:

•Semaglutide (GLP-1 RA): Approved for obesity/T2DM, reduces liver fat by 40% (31,32).

•Tirzepatide (GIP/GLP-1 RA): Superior weight loss and hepatic fat reduction vs. semaglutide (33).

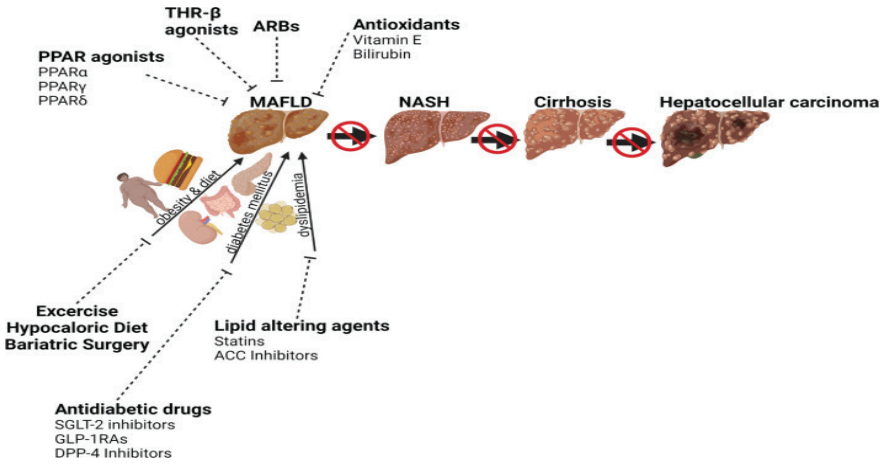


*Figure 4: Influence of current and emerging therapies on MAFLD.*

**B. Anti-inflammatory and Anti-fibrotic Agents**

1. Apoptosis Signal-Regulating Kinase 1 (ASK1) Inhibitors

Selonsertib: Phase 3 failure (34). Provided mechanistic insights into oxidative stress pathways. Next-generation candidates: More selective kinase inhibitors in development

2. Chemokine Receptor Antagonists

Cenicriviroc: Phase 2b CENTAUR trial showed fibrosis improvement(35). Failed to meet primary endpoint in Phase 3 AURORA study(36,37)

Emerging agents: Novel CCR2/5 inhibitors with improved pharmacokinetics

3. Galectin-3 Inhibition

Belapectin (GR-MD-02):

Ongoing Phase 3 NAVIGATE trial (NCT04365868)(38)

Primary endpoint: Fibrosis improvement without NASH worsening

**C. Nucleic Acid-Based Therapies**

1. Oligonucleotide Therapies

IONIS-PNPLA3Rx: Targets I148M variant (OR 3.5 for advanced fibrosis). Preclinical data show 60% hepatic fat reduction

ARO-HSD: RNAi targeting protective HSD17B13 variant. Phase 1/2 trial ongoing (NCT04202354)(39)

2. Gene Editing Approaches

CRISPR/Cas9: Preclinical PNPLA3/TM6SF2 editing shows promise. Delivery challenges remain for clinical translation

**D. Microbiome-Targeted Interventions**

1. Microbial Restoration Therapies

•FMT: Early-phase trials demonstrate metabolic improvement. Safety concerns require further evaluation

•Probiotics: Akkermansia muciniphila shows anti-steatotic effects. Phase 2 data pending for several formulations

2. Bile Acid Modulation

•NorUDCA: Phase 2 data show anti-fibrotic activity. Currently in Phase 3 development

•FXR Agonists: Obeticholic acid approved for PBC

•REGENERATE trial showed fibrosis benefit in NASH(41,42,43)

**E. Precision Medicine Approaches**

1. Genetic Stratification

•PNPLA3 I148M: Predicts poor response to weight loss

•HSD17B13: Modifies progression risk

•TM6SF2 E167K: Associated with atherogenic dyslipidemia

2. Multi-Omics Profiling

•Integration of: Metabolomics (lipid signatures)

•Proteomics (inflammatory markers)

•Transcriptomics (fibrosis pathways)(41,42,43)

*Table 3.Pharmacological Treatments in MAFLD: Agents and Mechanisms (2024)*

| Drug Class / Agent | Mechanism of Action | Clinical Stage / Notes |
|---|---|---|
| **FXR Agonists** | | |
| Obeticholic acid (OCA) | Activates farnesoid X receptor (FXR); ↓ bile acid synthesis, ↓ hepatic lipogenesis | Phase 3 (REGENERATE trial); improves fibrosis (41) |
| Tropifexor | Non-steroidal FXR agonist; ↑ insulin sensitivity, ↓ inflammation | Phase 2 (FLIGHT-FXR trial) (41) |
| **PPAR Agonists** | | |
| Lanifibranor | Pan-PPAR agonist (α/δ/γ); improves insulin sensitivity and reduces fibrosis | Phase 3 (NATiV3)(44) |
| Elafibranor | PPAR-α/δ agonist; reduces steatosis, inflammation, and fibrosis | Failed Phase 3 (RESOLVE-IT); discontinued(44) |
| Saroglitazar | Dual PPAR-α/γ agonist; improves lipid profile and insulin resistance | Approved in India for NASH(44) |
| **GLP-1 Receptor Agonists** | | |

| Drug Class / Agent | Mechanism of Action | Clinical Stage / Notes |
|---|---|---|
| Semaglutide | Enhances insulin secretion, ↓ appetite, promotes weight loss | Phase 2 showed steatohepatitis resolution (45) |
| Liraglutide | Similar GLP-1 RA; weight loss-mediated NASH improvement | Phase 2 (LEAN trial) |
| **Thyroid Hormone Receptor-β Agonists** | | |
| Resmetirom (MGL-3196) | THR-β agonist; ↑ hepatic fat oxidation, ↓ DNL | Phase 3 (MAESTRO-NASH); significant liver fat reduction (45) |
| VK2809 | Similar mechanism to Resmetirom; liver-targeted THR-β agonist | Phase 2 |
| **FASN Inhibitors** | | |
| TVB-2640 (Denifanstat) | Inhibits fatty acid synthase; ↓ DNL and oxidative stress | Phase 2(39) |
| **CCR2/CCR5 Antagonists** | | |
| Cenicriviroc | Blocks CCR2/5-mediated monocyte recruitment; anti-inflammatory and anti-fibrotic | Phase 3 failed (AURORA trial) (46) |
| **Mitochondrial Modulators** | | |
| MSDC-0602K | Targets mitochondrial pyruvate carrier; improves insulin sensitivity | Phase 2b(47) |
| **SGLT-2 Inhibitors** | | |
| Empagliflozin, Dapagliflozin | Promote glycosuria and weight loss; improve steatosis in diabetic MAFLD patients | Off-label use; under investigation (48) |

## 6. Conclusion

The MAFLD therapeutic landscape is rapidly evolving, with novel agents targeting metabolic, inflammatory, and fibrotic pathways. Precision medicine, guided by genetics and multi-omics, will shape future management. Clinical trials must prioritize hard endpoints (e.g., cirrhosis regression, HCC prevention).

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# Chapter 5

# MULTIDIMENSIONAL PERSPECTIVES ON DEMENTIA: ETIOLOGY, PROGRESSION, AND THERAPEUTIC APPROACHES

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## Introduction

Dementia is a growing global public health issue, especially among the aging population. It involves a gradual decline in cognitive functions, such as memory, attention, language, executive skills, and the ability to carry out daily tasks (World Health Organization [WHO], 2023). The condition not only affects individuals but also places emotional and social stress on caregivers (Prince et al., 2022). Currently, around 60 million people worldwide live with dementia, and this number is expected to rise to 139 million by 2050 (Long et al., 2023). Its prevalence increases with age, from 1-2% in those aged 65-69 to nearly 30% among individuals aged 85 and older (Prince et al., 2022). Rapid population aging, particularly in developing countries, adds further pressure to global healthcare systems (WHO, 2023). The medical understanding of dementia began to take shape after Alois Alzheimer's identification of its pathological features in 1906 (Lane, Hardy, & Schott, 2018). His discovery of amyloid beta plaques and neurofibrillary tangles in the brain of a patient with early-onset dementia laid the foundation for current research. Since then, advances have improved understanding of the disease's mechanisms, enabled earlier diagnosis, and supported the development of treatments. Alzheimer's disease is the most common form, responsible for 60-70% of all cases (WHO, 2023). Other subtypes include vascular dementia, Lewy body dementia, and frontotemporal dementia (Livingston et al., 2020). Alzheimer's is marked by the build-up of amyloid beta and tau proteins, while vascular dementia is linked to cerebrovascular damage (Lane et al., 2018; O'Brien & Thomas, 2015). Lewy body dementia involves abnormal protein deposits in the brain and often shares symptoms with Parkinson's disease (McKeith et al., 2017). Frontotemporal dementia typically presents with behavioral and language changes (Bang, Spina, & Miller, 2015). Both modifiable and non-modifiable risk factors contribute to dementia. These include age, genetics, cardiovascular diseases, hypertension, diabetes, obesity, smoking, low education levels, and chronic stress (Livingston et al., 2020). However, lifestyle changes such as regular physical activity, a healthy diet (like the Mediterranean diet), mental stimulation, and social engagement may help reduce risk (Livingston et al., 2020; WHO, 2023). Diagnosis is mainly based on clinical assessment, but biomarkers such as amyloid PET scans and cerebrospinal fluid tests now allow earlier and more accurate detection (Jack et al., 2018; Dubois et al., 2021). While there is no cure, medications like cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and NMDA receptor antagonists (memantine) help manage symptoms in Alzheimer's disease (Birks, 2006). Research into new treatments, including anti-amyloid drugs, is ongoing (Cummings et al., 2021). Non-drug interventions such as cognitive training,

physical activity, dietary support, and social interaction are also important in improving quality of life (Livingston et al., 2020). From a public health perspective, the rising dementia burden calls for coordinated strategies. These include raising awareness, promoting prevention, supporting early diagnosis, and improving access to care services (WHO, 2023).

**History of Dementia**

The term dementia originates from the Latin demens, meaning "without mind" or "mental disorder." While it began appearing in general use during the 13th century, its inclusion in medical literature did not occur until the 18th century (Assal, 2019; O'Brien, 2007). Scientific investigation into dementia began in the early 20th century. In 1901, German physician Alois Alzheimer examined a 51-year-old patient, Auguste Deter, who exhibited memory loss, disorientation, and impaired reading and writing. After her death in 1906, he studied her brain and identified unusual pathological features now known as amyloid plaques and neurofibrillary tangles. That same year, Alzheimer presented his findings in a lecture titled A peculiar disease of the cerebral cortex, and in 1907, he published a detailed article (Alzheimer, 1907). The condition was later named "Alzheimer's disease" by his mentor Emil Kraepelin in a psychiatry textbook published in 1910 (Maurer et al., 1997). By the mid-20th century, researchers had identified dementia as a condition with several subtypes, including Alzheimer's disease, vascular dementia, and Lewy body dementia. The development of neuroimaging techniques such as CT and MRI greatly improved the accuracy of diagnosis. (Alzola et al., 2024).

**Definition and Clinical Features of Dementia**

The term *dementia*, derived from the Latin mens ("mind"), was first introduced by Philippe Pinel as a decline in judgment and reasoning (Yang et al., 2016). Today, dementia is defined as a progressive neurodegenerative disorder that impairs memory, cognition, language, reasoning, and social function (Tariq & Barber, 2018). According to the WHO (2023), it is a chronic condition primarily affecting individuals over 65, interfering with daily functioning and leading to increased dependency (Alzheimer's Association, 2023). Although more prevalent in older adults, dementia can also occur in younger individuals and is not solely a feature of aging or limited to Alzheimer's disease (Carter, 2014). Diagnosis requires decline in at least one cognitive domain such as memory, attention, language, or social cognition that disrupts daily life and cannot be attributed to transient conditions like delirium. Common symptoms include memory loss, attention deficits, language issues, disorganized thinking, perceptual disturbances, and behavioral changes, with at least two domains

typically affected (Carter, 2014). Normal aging involves some cognitive decline due to brain changes like synaptic loss and neurotransmitter imbalance (Harada et al., 2013), but not all older adults develop dementia. Mild Cognitive Impairment (MCI) is considered a transitional phase, with 12-15% of individuals progressing to dementia annually (Petersen et al., 2014). Globally, about 50 million people live with dementia, projected to reach 152 million by 2050 (WHO, 2023). In Turkey, roughly 800,000 individuals are affected, with two-thirds diagnosed with Alzheimer's disease (Gurvit et al., 2008). Dementia has become a significant public health issue due to its progressive nature and broad cognitive impact. According to the Diagnostic Criteria for Dementia, memory impairment plus one of the following aphasia, apraxia, agnosia, or executive dysfunctions is required for diagnosis (Carter, 2014). The WHO (1992) further emphasizes decline in higher cortical functions and changes in emotional control, behavior, or motivation. Alzheimer's disease is the most common form, followed by vascular dementia, Lewy body dementia, and frontotemporal dementia (Geldmacher & Whitehouse, 1996). Other contributing conditions include cerebrovascular disease and Parkinson's disease (Hussain & Camicioli, 2018). Symptoms typically evolve from mild forgetfulness and disorganization to severe memory loss, functional impairment, and motor difficulties (Farooqui et al., 2019).

**Epidemiology of Dementia**

Rising global life expectancy and an expanding elderly population have driven a sharp increase in dementia cases (WHO, 2023). As of 2021, approximately 55 million people were living with dementia, with nearly 10 million new diagnoses annually roughly one every three seconds (Jain & Hogervorst, 2023). Over 60% of these cases occur in low- and middle-income countries (WHO, 2023). Dementia prevalence increases with age: it affects 2-10% of those under 65, 11% of people over 65, and 25-40% of individuals aged 90 and above (Prince et al., 2015; Hebert et al., 2013). In Turkey, around 800,000 individuals had dementia in 2019, with projections suggesting this could quadruple by 2050 (Gurvit et al., 2008). Globally, the number is expected to rise to 78 million by 2030 and exceed 150 million by 2050 (Guierchet, 2015). Both incidence and prevalence escalate with age. Incidence rises from 0.4% in those aged 65-69 to 10% among people in their 90s, while prevalence increases from about 2% in younger elderly to over 25% among nonagenarians (Burns & Iliffe, 2009; Prince et al., 2015). In high-income countries, 6-10% of those over 65 have moderate to severe dementia. Among individuals over 85, prevalence reaches 11% in men and 14% in women, and among those over 95, up to 36% and 41%, respectively (Hebert et al., 2013). Dementia is one of the most common neurodegenerative disorders and a major

cause of morbidity and mortality. A Swedish study reported a five-year mortality rate of 2.4% among patients aged 75+ (Smith & Ismail, 2021), while another found that 55% of care home residents with dementia died within 18 months, mainly due to malnutrition, pneumonia, and fever (Qiu & Fratiglioni, 2018). In Turkey, Alzheimer's affects about 11% of individuals aged 70+, with a related mortality rate of 3.9% (TÜİK, 2015; Aydın, 2020). WHO projected 57.4 million dementia cases in 2019, increasing to 83.2 million by 2030 and 153 million by 2050 (WHO, 2021; Türkiye Alzheimer Derneği, 2023). However, large-scale national data in Turkey are limited. One Istanbul-based study found a 20% dementia prevalence and 11% for Alzheimer's in those aged 70+ (Gürvit et al., 2008). Another study in Eskişehir reported 8.4% dementia prevalence in individuals aged 55+, with vascular dementia most common (Arslantaş et al., 2009). The economic burden is also substantial. In 2015, global dementia-related costs reached $818 billion, primarily impacting healthcare and long-term care systems (Prince et al., 2015). This underscores the urgent need for coordinated global strategies.

**Prevalence of Dementia**

The global shift toward an aging population is a major factor behind the rising prevalence of dementia (Prince et al., 2013). Epidemiologically, prevalence refers to the proportion of a population affected by a condition at a specific time. Systematic research on dementia prevalence began in the 1980s to better understand its public health impact (Hebert et al., 2013). As awareness increased, so did research, though reported rates still vary across regions due to differences in diagnostic criteria and methodology (Ferri et al., 2005). Studies of individuals aged 65 and over commonly report prevalence rates between 5% and 15%, with rates rising sharply with age (Prince et al., 2013). The World Alzheimer Report (2009) estimated 35.6 million global dementia cases, projected to reach 65.7 million by 2030 and 115.4 million by 2050 (Wimo, 2013). In Europe, the EuroDem and EuroCoDe projects estimated 6.8 to 7.3 million cases, with women more frequently affected (Prince et al., 2013). Prevalence increases from about 1.5% at age 65 to 22% at age 85+ (Peters et al., 2008). In high-income countries, around 10% of those aged 65+ and one-third of individuals over 85 have dementia (Hebert et al., 2013; Prince et al., 2013). In Turkey, roughly 20% of people aged 70+ are estimated to have dementia (Sharp & Gatz, 2011). Risk is influenced by age, gender, education, and socioeconomic status, with lower education linked to higher risk (Sharp & Gatz, 2011). Dementia prevalence doubles approximately every five years after age 60 (Prince et al., 2013). Prevalence is higher in institutional settings (50-54%) than among community-dwelling older adults (11.5-18.4%) (Hebert et al., 2013). WHO data from 2017 estimated 50 million

global cases, with 10 million new diagnoses each year, expected to reach 78 million by 2030 and 139 million by 2050 (WHO, 2019). Most cases (around 60%) are in low- and middle-income countries (WHO, 2019).

Prevalence also varies by region. East Asia reports the lowest rates, while South Asia has the highest (Li et al., 2022). Turkey, Bahrain, and Kuwait show relatively high prevalence, while India, Nigeria, and Pakistan report lower rates (Li et al., 2022). Women account for over 60% of dementia cases (Bae et al., 2018). In the U.S., the proportion of those aged 65+ rose from 4.1% in 1900 to 12.8% in 2000. About 5 million Americans had dementia in 2007, with projections of 13 million by 2050 (Hebert et al., 2013). Dementia ranks among the costliest health conditions in the U.S., with annual costs exceeding $100 billion (Hebert et al., 2013).

**Etiology of Dementia**

Dementia is a clinical condition marked by the gradual decline of cognitive abilities and is generally categorized into primary and secondary types. Primary dementias originate from intrinsic neurodegenerative processes directly impairing brain function, whereas secondary forms result from systemic conditions or external factors unrelated to primary brain pathology (Tesco & Lomoio, 2022). Alzheimer's disease (AD) is the most widespread among primary dementias, while vascular dementia is the leading secondary type (Kalaria, 2008; Tesco & Lomoio, 2022). Etiologically, Alzheimer's disease is responsible for roughly 60% of all dementia cases, with vascular causes accounting for an additional 10-20%. The remainder, approximately 20-30%, stems from diverse underlying conditions (Tesco & Lomoio, 2022). The most commonly encountered dementia subtypes include Alzheimer's disease, vascular dementia, Lewy body dementia, frontotemporal dementia, and HIV-related dementia. Less frequent causes include Parkinson's disease, Huntington's disease, Creutzfeldt-Jakob disease, and neurosyphilis (Carr, 2017). Recognizing potentially reversible causes of dementia is vital for guiding clinical interventions. These reversible factors encompass vitamin B12 deficiency, endocrine disorders (e.g., thyroid dysfunction, Cushing's syndrome), metabolic abnormalities (like electrolyte imbalances and hypoglycemia), infectious agents (e.g., HIV, syphilis), exposure to neurotoxins (such as alcohol and certain drugs), intracranial tumors, and psychiatric conditions (Kalaria, 2008; Carr, 2017). A wide range of both genetic and environmental influences contribute to the risk of developing dementia. Among these, advanced age stands out as the most critical determinant, with prevalence rising substantially beyond the age of 80 (Sahathevan, 2015). Additional key risk factors include being female, familial genetic variants (notably the apolipoprotein E-4 allele and mutations on chromo-

somes 1, 14, 19, and 21), Down syndrome, lower educational levels, traumatic brain injury, chronic exposure to neurotoxins, vascular conditions, hypertension, hyperlipidemia, elevated homocysteine, persistent inflammation, estrogen deficiency after menopause, tobacco use, and diabetes mellitus (Christopher, 2023). From an etiological standpoint, dementia can be divided into several subtypes: degenerative, vascular, infectious, neoplastic, traumatic, demyelinating, inflammatory, toxic, metabolic, and psychiatric. Alzheimer's disease is the most frequent among degenerative forms, followed by disorders like frontotemporal dementia, Huntington's disease, Lewy body dementia, and Pick's disease (Kowa, 2018). Vascular dementia is typically associated with cerebrovascular pathology and may coexist with Alzheimer's disease (Carr, 2017). The underlying pathophysiology of dementia involves a complex interaction between genetic vulnerabilities and modifiable environmental factors. For example, the presence of the APOE-4 allele significantly increases susceptibility to Alzheimer's disease, while vascular dementia is strongly linked to hypertension, cardiovascular issues, and diabetes. Other contributing mechanisms include oxidative stress, chronic inflammation, hormonal shifts, and poor dietary habits (Kalaria et al., 2008; Sahathevan, 2015). As the global population continues to age, dementia has become an urgent public health concern. Currently, around 55 million people are affected worldwide, and projections estimate this figure will escalate to 152 million by 2050, particularly in low- and middle-income regions. The economic impact is immense, with global dementia-related costs approaching 1 trillion USD annually (Evans et al., 2024). Consequently, prioritizing preventive measures such as managing risk factors and promoting healthy lifestyle practices is essential in addressing the growing dementia burden (Carr, 2017; Sahathevan, 2015).

**Risk Factors for Developing Dementia**

Early detection of dementia plays a crucial role in influencing the progression of the condition. Hence, gaining a comprehensive understanding of dementia risk factors is vital for both prevention strategies and effective clinical management. Dementia, and Alzheimer's disease (AD) in particular, rarely arises from a single cause; rather, it results from the complex interplay between various genetic predispositions and environmental exposures (As, 2023; Livingston et al., 2020). Generally, dementia risk factors are divided into two broad groups: those that are non-modifiable and those that can be altered or managed (Livingston et al., 2020; WHO, 2023).

### *Non-Modifiable Risk Factors*

Advanced age remains the most prominent and widely recognized risk factor for Alzheimer's disease (AD) and other dementia subtypes (Livingston et al., 2020; Prince et al., 2013). Over 90% of dementia diagnoses occur in individuals aged 65 and older, with risk escalating sharply as age advances (Prince et al., 2013). Genetic influences are also significant. In early-onset familial AD, mutations in genes such as amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) have been identified, whereas the ε4 variant of the apolipoprotein E (ApoE) gene is a well-established risk factor for late-onset (sporadic) AD (Karch & Goate, 2015; Corder et al., 1993). A positive family history of dementia, as well as genetic conditions like Down syndrome, further increase susceptibility (Lott & Head, 2019). In frontotemporal dementia (FTD), mutations inherited in an autosomal dominant manner, particularly in the MAPT, GRN, and C9orf72 genes, are frequently observed (Bang et al., 2015). Female sex is considered another non-modifiable risk factor, especially among the elderly, with women more commonly affected by AD than men a difference attributed to factors such as greater longevity and biological variations (Mielke, 2018; Alzheimer's Association, 2023). Additionally, ethnicity appears to impact dementia risk, with higher prevalence rates reported among Black African, Caribbean, and South Asian populations relative to White populations (World Health Organization [WHO], 2023).

### *Modifiable Risk Factors*

According to the Lancet Commission's 2020 and 2024 reports, a total of 14 modifiable risk factors contributing to dementia have been identified (Livingston et al., 2020; Livingston et al., 2024). These include factors such as limited educational attainment, head trauma, physical inactivity, smoking, excessive alcohol consumption, hypertension, obesity, diabetes, hearing and vision impairments, depression, social isolation, air pollution exposure, and elevated cholesterol. Educational attainment is particularly influential in fostering cognitive reserve, which acts as a buffer against the development of dementia. Numerous studies have linked higher education levels with improved cognitive performance in old age and a lower incidence of dementia (Kim, 2024). Additionally, traumatic brain injury (TBI) has been found to accelerate the onset and progression of Alzheimer's disease, partly by promoting post-injury β-amyloid accumulation (Smolen, 2023). Engaging in regular physical activity has neuroprotective benefits that slow cognitive deterioration; particularly, exercise during midlife mitigates cortical gray matter loss and lowers dementia risk (Jackson, 2016). Both smoking and heavy alcohol consumption contribute to dementia through mechanisms such as oxidative dam-

age, neuroinflammation, and direct toxic effects on neurons (Reale, 2020). Cardiometabolic factors including chronic hypertension, obesity, and diabetes are strongly implicated in dementia development. Hypertension promotes cognitive decline through pathways involving oxidative stress, inflammation, and autonomic nervous system dysfunction (Patel & Edison, 2024). Obesity contributes to neurodegeneration by fostering insulin resistance and systemic inflammation, whereas type 2 diabetes results in damage to peripheral and central nervous tissues (Biessels & Reagan, 2015). Sensory impairments, including hearing and vision loss, can exacerbate social isolation and diminish cognitive reserve, thereby increasing the risk of dementia (Erichsen et al., 2022). Psychological factors such as depression and social isolation adversely impact both brain function and cardiovascular health, facilitating dementia pathogenesis (Ownby et al., 2006). Additionally, exposure to air pollution affects brain health by inducing inflammatory responses and oxidative stress (Calderón-Garcidueñas et al., 2016).

### *The Importance of Risk Factors and Prevention*

Managing lifestyle and environmental risk factors presents a crucial opportunity to prevent or postpone the development of dementia. Livingston et al. (2020) estimate that around 40-45% of dementia cases worldwide are attributable to factors that can be modified. This highlights the importance of early preventive measures and adopting healthier lifestyle habits in mitigating the overall impact of dementia on a global scale (Livingston et al., 2020).

## Symptoms of Dementia

Dementia refers to a collection of progressive neurodegenerative disorders primarily characterized by the deterioration of cognitive functions over time. In addition to these cognitive impairments, behavioral and psychological symptoms of dementia (BPSD) form an essential component of the clinical presentation and significantly influence both diagnosis and therapeutic strategies. BPSD includes a wide array of non-cognitive manifestations such as agitation, aggression, depressive symptoms, anxiety, hallucinations, delusions, irritability, sleep disturbances, and apathy. Research indicates that nearly 90% of dementia patients experience at least one of these symptoms at some point during disease progression (Cerejeira et al., 2012). These symptoms not only diminish the quality of life of those affected but also impose substantial emotional, physical, and financial burdens on caregivers, thereby complicating caregiving efforts (Kales et al., 2015). For instance, depression and apathy often contribute to social withdrawal and isolation, while agitation and aggression are

commonly linked to elevated caregiver distress and burnout. Moreover, the emergence of BPSD is associated with earlier institutionalization, increased dependence on antipsychotic medication, and overall worse clinical outcomes (Kales et al., 2015; Cerejeira et al., 2012). Managing BPSD effectively is therefore vital to delivering holistic dementia care. Nonetheless, these symptoms are often underdiagnosed or misunderstood. Research shows that both professional and informal caregivers frequently lack adequate knowledge and confidence in addressing BPSD (Bessey & Walaszek, 2019). This deficiency contributes to the underuse of recommended non-pharmacological treatments, such as behavioral interventions, environmental adjustments, and music therapy, which are considered first-line approaches for managing BPSD (Livingston et al., 2017). In recent years, several assessment tools have been created to gauge caregivers' dementia-related knowledge; among these, the Dementia Knowledge Assessment Scale (DKAS) is the most employed. However, it primarily targets cognitive symptoms and falls short in adequately evaluating understanding of behavioral and psychological symptoms (Hu et al., 2023). This shortcoming poses a significant challenge, especially when designing educational programs tailored to specific symptom domains. Furthermore, the scarcity of validated instruments dedicated to measuring knowledge of BPSD hampers both clinical practice and caregiver education initiatives. The absence of culturally adapted and psychometrically robust tools in languages other than English further widens this gap in nations such as Turkey, where dementia care infrastructure is still developing (Uysal et al., 2024). Consequently, improving the awareness and expertise of caregivers and healthcare professionals in the identification, evaluation, and management of BPSD represents an urgent priority.

**Pathologically Based Classification and Subtypes of Dementia**

The most precise way to classify dementia is grounded in its fundamental pathological processes. This approach primarily depends on identifying abnormal protein aggregations within neurons and glial cells. These pathological inclusions disrupt normal cellular and molecular functions, leading to impaired cell viability and potentially causing neuronal death. Consequently, widespread neural networks become dysfunctional, producing various impairments across cognitive, behavioral, and sensorimotor domains. The particular brain regions affected, and the temporal progression of these dysfunctions offer important clues for clinicians to understand the disease's underlying pathology (Elahi & Miller, 2017). From an etiological standpoint, dementia is generally divided into two principal categories: neurodegenerative and non-neurodegenerative origins. Neurodegenerative forms, including Alzheimer's disease, frontotemporal dementia, and dementia with Lewy bodies, are predominantly

found in older populations. Conversely, non-neurodegenerative dementias resulting from vascular incidents, infections, metabolic imbalances, or traumatic brain injuries are more frequently encountered in younger and middle-aged individuals (Hendriks et al., 2021). The diagnosis of dementia involves a thorough clinical assessment encompassing a detailed medical history, physical examination, laboratory investigations, and the recognition of distinctive deficits in cognitive and executive functions (Dickerson & Atri, 2025). Nonetheless, identifying the exact dementia subtype presents greater complexity than establishing the general diagnosis. This challenge is largely due to the overlapping symptoms among various dementia types and the individual differences in how symptoms manifest and progress over time (Dickerson & Atri, 2025).

### *1. Alzheimer's Disease (AD)*

Alzheimer's disease represents the leading cause of dementia, accounting for roughly 60-80% of cases (Dickerson & Atri, 2025). It is a progressive neurodegenerative disorder distinct from normal aging, though its prevalence rises with increasing age. The defining pathological characteristics of Alzheimer's disease include the extracellular deposition of beta-amyloid plaques, and the intracellular accumulation of neurofibrillary tangles composed of hyperphosphorylated tau protein. These abnormalities result in neuronal degeneration and extensive brain tissue damage (Dickerson & Atri, 2025). The disease generally originates in brain areas associated with learning and memory. Initial clinical signs often involve difficulties with recalling names and recent events, alongside symptoms such as apathy and depression. As the condition advances, patients experience profound cognitive and physical decline, including disorientation, behavioral disturbances, paranoia, hallucinations, and challenges with speech and swallowing. Frequently, patients lack awareness of these deficits, which are typically first observed by relatives or primary caregivers (Dickerson & Atri, 2025).

### *2. Vascular Dementia*

Vascular dementia arises from cognitive decline caused by blockages, tissue death, or hemorrhagic incidents impacting cerebral blood vessels. The intensity of clinical symptoms varies according to the number, size, and anatomical location of brain lesions. Representing about 5–10% of dementia cases, vascular dementia primarily affects executive functions such as decision-making, planning, and organizational abilities. Key risk factors include stroke, hypertension, atherosclerosis, diabetes mellitus, and tobacco use (Stefanova & Kostić, 2016). Its prevalence escalates with age, increasing from around 1% in individuals aged 55 to 4.2% by age

71. Moreover, vascular dementia occurs more frequently in East Asian populations compared to Alzheimer's disease (Stefanova & Kostić, 2016).

***4. Dementia with Lewy Bodies (DLB)***

Dementia with Lewy bodies (DLB) is caused by the abnormal build-up of alpha-synuclein proteins inside neurons. Representing about 5% of dementia diagnoses, DLB frequently coexists with Alzheimer's disease (Kane, 2018). Distinctive clinical manifestations of DLB include visual hallucinations, fluctuating attention levels, sleep disorders, and motor symptoms resembling Parkinson's disease. Memory deficits usually appear during the later phases and tend to be less severe compared to Alzheimer's disease. Additionally, symptoms in DLB tend to vary significantly throughout the day (Simpson, 2025).

***5. Frontotemporal Dementia (FTD)***

Frontotemporal dementia (FTD) comprises a group of neurodegenerative disorders characterized by progressive neuronal loss predominantly affecting the frontal and temporal lobes of the brain. Damage in these regions results in noticeable changes in personality, disturbances in social conduct, and language-related difficulties. FTD typically manifests between the ages of 40 and 60 and is recognized as the second most common form of dementia among individuals under 65 years of age (Young et al., 2018). The pathological hallmarks of FTD involve abnormal accumulation of proteins such as tau and TDP-43. In contrast to Alzheimer's disease, early stages of FTD are more frequently marked by behavioral and linguistic impairments, rather than memory deficits. Moreover, hallucinations are rarely observed in FTD, which distinguishes it from later stages of Alzheimer's disease where such symptoms are more prevalent. Currently, there is no curative treatment for FTD, and as the disease advances, patients often experience severe functional decline, including motor impairments and complete dependence on caregivers (Young et al., 2018).

***6. Parkinson's Disease Dementia (PDD)***

Parkinson's disease dementia (PDD) is characterized by cognitive deficits that arise at least one year following an initial Parkinson's disease diagnosis. Representing about 10% of dementia cases, PDD symptoms include impaired decision-making, visual disturbances, memory deficits, reduced speech volume (hypophonia), hallucinations, and REM sleep behavior disorder. The hallmark pathology involves extensive alpha-synuclein aggregation throughout the brain, which contributes to cognitive deterioration alongside the classic motor symptoms. As the condition ad-

vances, difficulties with attention, executive functioning, and planning emerge, significantly impacting daily activities (Kane et al., 2018).

## Current Approaches in the Treatment of Dementia

Dementia is widely acknowledged as a significant global public health challenge, with its prevalence steadily rising. To date, no therapies exist that can completely stop the disease's progression or provide a cure. Treatment primarily focuses on alleviating cognitive deficits, managing mood and behavioral disturbances, slowing the course of the illness, and enhancing patients' overall quality of life (Chandler et al., 2024).

### *Pharmacological Approaches*

Pharmacological treatments for dementia focus on supporting cognitive abilities, addressing behavioral symptoms, and decelerating disease progression. The U.S. Food and Drug Administration (FDA) has approved five medications: donepezil, rivastigmine, galantamine, tacrine, and memantine. Among these, donepezil, rivastigmine, galantamine, and tacrine function as acetylcholinesterase inhibitors, whereas memantine acts as an NMDA receptor antagonist (Govind, 2020). Acetylcholinesterase inhibitors work by elevating acetylcholine levels in the brain, thereby enhancing synaptic communication. In contrast, NMDA antagonists aim to mitigate glutamate-induced neurotoxicity, a neurotransmitter involved in memory and learning processes (Puranik & Song, 2024; Govind, 2020). Clinically, acetylcholinesterase inhibitors are predominantly utilized, particularly for Alzheimer's disease (Ortner et al., 2020). While these agents may be prescribed at various stages of Alzheimer's, memantine demonstrates greater efficacy in moderate to severe phases. Additionally, managing vascular dementia often involves medications targeting blood pressure and cholesterol to reduce further cerebral damage (WHO, 2023). To address neuropsychiatric manifestations such as agitation, aggression, hallucinations, and anxiety, treatment regimens may also include antipsychotics, antidepressants, anxiolytics, and anticonvulsants (Ohno et al., 2006). In cases of dementia with Lewy bodies and Parkinson's disease dementia, rivastigmine and donepezil have shown cognitive benefits. Moreover, drugs like lorazepam, clonazepam, and melatonin have been reported to alleviate symptoms of REM sleep behavior disorder (Mori et al., 2012).

### *Non-Pharmacological Approaches*

Non-pharmacological interventions are vital in symptom management and improving the quality of life for people living with dementia. These approaches encompass cognitive rehabilitation, adjustments to the envi-

ronment, psychosocial support, lifestyle changes, and various complementary therapies. Cognitive rehabilitation focuses on helping patients retain daily functional abilities and enhancing their capacity, especially during the initial stages of dementia (Kasper et al., 2015). Engagements such as reading, mental games, cognitive exercises, and reminiscence therapy have demonstrated benefits in maintaining cognitive abilities (Arvanitakis et al., 2019). Nonetheless, it is crucial to avoid activities that are excessively challenging, as they may induce frustration or stress. Environmental adaptations and assistive technologies contribute to preserving patient independence by establishing safer and more supportive living conditions (Tournoy et al., 2022). Psychosocial interventions strengthen emotional resilience and lessen caregiver stress through educational programs and support groups aimed at both patients and their caregivers. Physical activity is another effective element in dementia care, with aerobic exercises like walking and swimming, as well as resistance training, linked to cognitive improvements (Wang et al., 2021). Lifestyle modifications, including sleep hygiene education, have shown efficacy in reducing symptoms of depression and sleep disorders (28). Nutritional strategies are also integral to dementia management. Diets such as the Mediterranean diet and those high in polyunsaturated fatty acids promote neuroprotection by enhancing synaptic plasticity and neuronal health (Ristori et al., 2025). Additionally, ketogenic diets have yielded positive results, particularly in individuals lacking the apolipoprotein E4 (apoE4) gene variant (Lilamand et al., 2020).

### *Importance of a Multidisciplinary Approach*

Implementing a multidisciplinary strategy is crucial to optimize dementia care. Coordinated efforts among healthcare professionals including physicians, dietitians, social workers, geriatric nurses, and speech-language pathologists facilitate the creation of personalized care plans and enhance patient outcomes (Grand et al., 2011).

## Dementia and Quality of Life

Quality of life is a complex, multidimensional construct that incorporates both subjective and objective evaluations of various life domains. This concept reflects an individual's overall well-being and encompasses factors such as mood, life satisfaction, emotional experiences, and contentment with social relationships and occupational roles (Gonzales-Salvador et al., 2000). According to the World Health Organization, quality of life is defined as a person's perception of their position within the cultural and value systems they inhabit, alongside their goals, expectations, physical and mental health, social interactions, and environmental

context. Health-related quality of life more specifically pertains to how health conditions including illnesses, treatments, or disabilities affect an individual's appraisal of their life (Ready & Ott, 2003). This perception is known to vary considerably with age, influenced directly by aging itself and indirectly by the resulting social, economic, and functional changes (Marventano et al., 2015). Dementia, a neurodegenerative disorder marked by progressive cognitive decline that often worsens with advancing age, significantly compromises quality of life. Impairments affecting self-care, social connectivity, and engagement in meaningful pursuits are common and contribute negatively to overall well-being in affected individuals (Kerpershoek et al., 2018). Additionally, the quality of life of informal caregivers tends to deteriorate alongside the progression of dementia (Banerjee et al., 2009). Evaluating quality of life is therefore critical for appreciating the personal impact of dementia and guiding patient-centred care approaches. However, no universally accepted gold standard exists for these assessments, and there remains debate in the literature about which factors most strongly influence quality of life (Bowling et al., 2015). Progressive cognitive impairment in dementia can reduce patients' awareness of their condition, often leading to overestimations of their well-being (Smith et al., 2005). This underscores the importance of incorporating caregiver perspectives alongside self-reports in quality-of-life evaluations. Nevertheless, quality of life is an inherently subjective and individualized experience, making the inclusion of patients' self-assessments essential, even when cognitive deficits are present (Brod et al., 1999; Higginson & Carr, 2001). Ensuring that individuals with dementia are actively involved in decisions about their care fosters empowerment and participation, which in turn has been linked to improved quality of life (Moyle et al., 2011). Efforts to enhance quality of life in dementia emphasize not only personalized care but also broader social interventions, such as cognitive stimulation, community engagement, and support networks (Xing et al., 2024). Furthermore, identifying populations experiencing both significant economic strain and diminished quality of life is vital for shaping health policies that promote effective resource allocation and equitable healthcare delivery (Xing et al., 2024).

**Phytotherapeutic Treatment Approaches in Dementia**

Although scientific interest in medicinal plants and herbal therapies for dementia has grown substantially over the last 15 years, traditional knowledge on this subject extends back centuries, if not millennia. Fields such as ethnopharmacology and archaeopharmacology provide insights into how diverse cultures identified and transmitted the therapeutic properties of plants through generations. Established medical traditions like Traditional Chinese Medicine (TCM) and European herbalism offer

valuable information concerning the safety and efficacy of these botanical treatments. The enduring use of such herbs throughout history implies that, when correctly applied with appropriate plant parts, preparation techniques, and dosages, they are generally safe and well tolerated. Herbal medicines exhibit a range of mechanisms due to their complex mixtures of chemical constituents and varied biological effects, making them increasingly attractive for contemporary pharmacological research. Moreover, medicinal plants remain a vital source for novel drug discovery. Numerous pharmacological agents currently in use are derived from plants, including ephedrine (Ephedra sinica), hyoscine (Hyoscyamus niger), morphine (Papaver somniferum), physostigmine (Physostigma venenosum), and galantamine (Galanthus and Narcissus species). Research into new plant-derived pharmaceuticals continues actively in various fields such as oncology (Adams et al., 2007; Man et al., 2010), infectious diseases, pain management (Liu et al., 2021), and immune or inflammatory conditions. The influence of phytochemicals on complex neurological processes like cognition and mood is believed to have evolved from plant compounds designed to interact with the nervous systems of animals for defensive or reproductive purposes for example, deterring herbivores or attracting pollinators and seed dispersers. These neuroactive properties are also exploited in insecticide development; pyrethrins from chrysanthemums, for instance, act on sodium channels to produce insecticidal effects (Palmquist, 2012). The rationale supporting the potential role of herbal medicines in future dementia treatments is grounded in three key trends: (1) the rising prevalence and economic impact of dementia, (2) the slow advancement in synthetic drug development due to disease complexity and rigorous regulatory requirements, and (3) increasing public interest in complementary and alternative medicine modalities (Man et al., 2008). Phytotherapeutic strategies have been widely examined within the scientific literature. Adams et al. (2007) documented the use of over 150 plant species aimed at alleviating age-related cognitive decline. Among those plants recognized for their acetylcholinesterase (AChE) inhibitory properties, notable examples include Salvia, Melissa officinalis, Huperzia serrata, and Ginkgo biloba (Mantle et al., 2004). Furthermore, phenolic compounds such as resveratrol (present in grapes and red wine), curcumin (derived from turmeric), and epigallocatechin (found in green tea) have been highlighted for their neuroprotective potential, particularly through mechanisms combating oxidative stress (Huang et al., 2008). According to Man et al. (2000), herbal treatments may offer safe and effective options for Alzheimer's disease when validated by rigorously controlled clinical studies. Similarly, Hyde et al. (2017) reported largely favorable findings supporting this perspective.

Non-pharmacological Approaches and Aromatherapy

The growing recognition of non-pharmacological methods in addressing behavioral symptoms in dementia patients has been well documented (Abraha et al., 2017). These interventions are typically divided into four primary categories:

- Cognitive-behavioral orientation (e.g., reality orientation),
- Sensory stimulation (e.g., acupuncture, aromatherapy, light therapy, touch therapy),
- Behavioral management,
- Psychosocial techniques (e.g., animal-assisted therapy, exercise).

Aromatherapy has attracted notable interest among non-pharmacological treatments. Archaeological findings suggest that essential oils were used as far back as 50,000 BC, and Hippocrates provided detailed descriptions of medicinal plants between 460 and 377 BC (Agnihotry et al., 2024). Aromatherapy involves therapeutic use of fragrant compounds and can be delivered through various methods such as massage, inhalation, ambient diffusion, baths, compresses, or mouth rinses. Essential oils like Melissa officinalis (lemon balm) and Lavandula angustifolia (lavender) are frequently utilized in dementia care, not only for their calming properties but also for cognitive benefits (Ballard et al., 2002). A few studies have shown that aromatherapy can reduce agitation and behavioral issues in dementia patients. For instance, Smallwood et al. (2001) found that massage combined with lavender oil significantly alleviated behavioral symptoms, while Lin et al. (2007) reported that a 10-week course of lavender oil inhalation decreased agitation levels. Nevertheless, some researchers, including Forrester et al. (2014), argue that conclusive evidence on the effectiveness of aromatherapy is limited, mainly due to small study sample sizes.

**Conclusion**

Dementia is a multifaceted neurodegenerative syndrome that profoundly impairs individuals' quality of life and poses significant challenges to healthcare systems on a global scale. Although cognitive deterioration associated with aging has been observed since antiquity, dementia only received formal clinical recognition in the early 20th century, following Alois Alzheimer's seminal pathological findings that led to the identification of Alzheimer's disease as the first distinct subtype (Lane, Hardy & Schott, 2018). The condition is typically characterized by a progressive decline in domains such as memory, language, executive func-

tions, attention, spatial awareness, and orientation, often culminating in a loss of autonomy in everyday activities (McKhann et al., 2011). Diagnosis involves a combination of clinical assessment, cognitive testing, neuroimaging, and biomarker analysis. As of 2023, approximately 60 million people globally are affected by dementia, with projections indicating this figure may surpass 120 million by 2050 (WHO, 2023). While aging is the predominant risk factor, numerous modifiable contributors are also implicated. For instance, Livingston et al. (2020) estimated that as many as 40% of dementia cases could be delayed or prevented by addressing factors like cognitive engagement, cardiovascular health, and hearing preservation. The etiology of dementia is complex, involving both non-modifiable risks, such as age, biological sex, genetics (e.g., APOE ε4 allele), and family history, and modifiable risks, including hypertension, type 2 diabetes, obesity, inactivity, smoking, depression, and social disconnection (Gauthier et al., 2022; Livingston et al., 2020). Dementia encompasses several clinical variants, each with unique pathophysiological and clinical features. Alzheimer's disease is typified by amyloid-beta deposition and tau protein tangles, whereas vascular dementia arises from cerebrovascular compromise (O'Brien & Thomas, 2015). Lewy body dementia is associated with motor disturbances and visual hallucinations, while frontotemporal dementia predominantly affects behavior and language capabilities (Bang et al., 2015). Parkinson's disease dementia, on the other hand, typically emerges during the later stages of Parkinson's disease progression (McKeith et al., 2017). Although a definitive cure remains elusive, several pharmacological treatments are employed to manage symptoms. Cholinesterase inhibitors (e.g., donepezil, rivastigmine, galantamine) and NMDA receptor antagonists (such as memantine) are standard therapies in Alzheimer's management (Birks, 2006). Recently, anti-amyloid monoclonal antibodies like lecanemab and aducanumab have gained FDA approval, although their efficacy and safety profiles are still under scrutiny (Cummings et al., 2021). Beyond drug-based interventions, non-pharmacological strategies play a vital role in enhancing patient well-being and alleviating caregiver burden. These include cognitive rehabilitation, physical activity, music and sensory therapies, and social participation programs (Olazarán et al., 2010). A multidisciplinary approach is essential for addressing both physical symptoms and psychosocial needs. Interest in complementary and alternative therapies is also growing. Phytotherapeutic and aromatherapeutic agents such as Melissa officinalis, Ginkgo biloba, and Lavandula angustifolia have shown promise for their neuroprotective and anxiolytic properties (Kennedy et al., 2003; Ernst, 2007). However, these interventions should be integrated cautiously with conventional treatment regimens, with careful attention to individual responses and potential interactions.

In conclusion, dementia represents a critical global health challenge with wide-ranging social, personal, and economic implications. Tackling this issue requires an integrated strategy focused on early detection, prevention, and multimodal treatment approaches. Future advancements in genomics, artificial intelligence, and precision medicine offer hope, but sustained progress depends on increased public awareness, equitable access to healthcare, and the development of robust care infrastructure.

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# Chapter 6

# PHYTOCHEMICAL PROFILE AND BIOMEDICAL SIGNIFICANCE OF ARUM MACULATUM L.

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## 1.Introduction

Nutrition is the intake of the energy and the nutrients that people need to be healthy and dynamic individuals in the their daily lives. The environment in the which people live is among the main factors that determine their diet. Adapting to the ecosystem, human beings have created their own culinary tradition by creating their diet according to the environmental conditions. While the plants are consumed as nutrients, the information gained as a result of the past experiences and observations have been used for the purpose of both the nutrition and for therapeutic purposes of the plants (Ceylan & Akar, 2022). It is estimated that there are 750.000-1.000.000 plant genus in the world. The biochemical structure of approximately 500.000 of the plant genus has been illuminated. It is stated that all the plants that are consumed for the nutritional purposes worldwide are around 20% of all plants.

From the Araceae family, perennial herbaceous species *Arum maculatum* L. mostly found in humid woodland settings all around Europe and Western Asia. Its unusual chemical composition and physical characteristics have attracted much of botanical and pharmacological interest. Though its poisonous qualities, this plant has been used in both ornamental horticulture and folk medicine under common names including “snake bread,” “wild chard,” “spotted snake pillow,” “heaven’s staff,” and others (Huxley et al., 1992).

It is the proved by scientific studies that *A. maculatum* plant of the contains many substances. The substances such as the glycoside and saponin in the plant content give the arum maculatum plant a toxic feature. Saponins an alternative to antibiotics, is a herbal ingredient studied on the phytochemicals. Having of the antibiotic and antifungal activities up made saponin content more important. This herbal ingredient, which is the contains high levels of the saponins and can be consumed by very few people and animals due to it is bitter taste plays an important role in the preventing colon cancer. The lectin substance in the content of the *A. maculatum* has brought the plant anti-inflammatory properties. It has been experimentally and clinically proven that lectin substance has anti-inflammatory activity in the enteron and respiratory system (Ceylan & Akar, 2022). Vitamins C and E are high in the structure of the *A. maculatum* plant. Has the antioxidant properties *A. maculatum* plant and is also an abundant source of the protein. In the traditional public health the *A. maculatum* plant is the predicted to treat intestinal cleanser and rheumatic pain in the cleaner of colon, liver disease, kidney stone disease and hyperactivity.

## 2.Features of Morphology

The arrow-shaped (sagittate) leaves of this species emerge in the spring from a rhizomatous base. Elongated petioles support these glossy, occasionally mottled leaves that emerge straight from the rhizome (Mabberley, 2008).

This plant is unique in its reproductive structure: a central spadix (a spike-like inflorescence) is encased in a large spathe (a bract that resembles a sepal). The tube-like, typically greenish to purple spathe encircles the spadix, which is separated into three functional zones: male flowers at the top, sterile flowers in the middle, and female flowers at the lower end (Stejskal, 2005). The spadix can generate metabolic heat during blooming, which draws insects and facilitates efficient pollination (Seymour et al., 2003). The plant produces eye-catching clusters of vibrantly colored berries by late summer. These fruits may seem beautiful, but they contain poisonous substances that can harm both people and animals.

**Figure 1:** *A. maculatum structural details illustrating the spathe and spadix (Petruzzello, 2018).*

## 3.Ecological Features and Habitat

The species *A. maculatum* is suited to environments that are damp and shaded. Its natural range includes parts of North Africa, Western Asia, and western to eastern Europe (Meusel et al., 1978). It is common in Turkey's shrublands and moist forest undergrowth, particularly in the Marmara and Black Sea regions (Davis, 1984). It can compete with other herbaceous species as forest undergrowth and prefers well-drained, humus-rich soils.

The plant grows quickly in the spring, loses its leaves in the middle of the summer, and goes into dormancy in underground rhizomes.

### 4.Traditional Use and Ethnobotanical Significance

*A. maculatum* has long been used in folk medicine-especially in Europe-to treat a range of ailments. Ground powder from its rhizomes has been used orally or topically to treat coughs, asthma, rheumatism, and warts (Grieve, 1931). But because of their high concentrations of calcium oxalate crystals and saponins, all of the plant-especially fresh-parts are poisonous. Thus, used improperly, it can produce symptoms including burning in the mouth, difficulty swallowing, nausea, vomiting, and, in severe cases, respiratory depression (Cooper & Johnson, 1984).

Furthermore noteworthy is the plant's starch concentration. Starch used as a food additive under the name "Portland arrowroot" or in baby food was obtained historically by drying the rhizomes. But in view of current toxicological knowledge (Turner & Szczawinski, 1991), this practice has been dropped.

### 5.Phylogenetic and Taxonomic Location

Comprising almost 120 genera and more than 3750 species, the Araceae family Arum is the genus that comprises almost 25 known species within this family (Boyce, 2006). Particularly *A. maculatum* shows great morphological variation which results in its classification as a varietal form or subspecies in some geographical areas (Lack & Kühn, 1984). Supporting its unique evolutionary placement, molecular phylogenetic studies show that this species belongs to a monophyletic lineage (Cusimano et al., 2011).

**Figure 2:** *A. maculatum natural occurrence in a forested ecosystem (Moro, 2025).*

## 6.Phytochemical Composition and Molecular Structures

*A. maculatum* is representative of the Araceae members with diverse and intricate phytochemical composition. The plant, which is rich in secondary metabolites, utilizes the molecules to a significant extent in responding to environmental stress and herbivory defense.

Calcium oxalate crystals, saponins, flavonoids, and alkaloids are some of the bioactive substances found in tissues such as rhizomes, roots, and leaves. Additionally, modulating the toxicological profile of the species as well as its alleged therapeutic advantages are these substances.

### 6.1.Calcium Oxalate Crystals

Typically needle-shaped in form, *A. maculatum* has a very notable anatomical characteristic in the abundance of calcium oxalate crystals. These crystals deter herbivory through mechanical injury and a defense function. Restricted to intracellular vacuoles, they are very irritating and stinging whenever they make contact with mucosal membranes that of the lip, the tongue, or the throat. Chemically, such structures typically appear as monohydrate or dihydrate calcium oxalate (Franceschi & Nakata, 2005).

### 6.2.Alkaloids

It also produces several nitrogen-containing substances known as alkaloids that are neuroactive and antibacterial in character. Review of the literature reports structurally similar tropane and pyrrolidine ring alkaloids. Such drugs may disrupt neural function, manifesting as vertigo, nausea, hallucinations, and incoordination (Fujii et al., 2007).

### 6.3.Flavonoids

Another main group of metabolites in *A. maculatum* are strong antioxidant flavonoids, phenolic compounds. Several flavonoid subtypes have been found by research; most of them are glycosides of flavonols and flavones (Harborne & Williams, 2000). Mostly found in floral and foliar tissues, these molecules help to UV protection, anti-inflammatory action, and free radical neutralizing effect by means of their C6-C3-C6 backbone and structural definition.

### 6.4. Saponins

Involved in plant defense, saponins-triterpenoid or steroidal in character-are surface-active molecules. These molecules have been demonstrated in *A. maculatum* to affect the permeability of cellular membranes in

both animal and bacterial cells, so producing either toxic or antimicrobial effects (Hostettmann & Marston, 1995). Their detergent-like qualities call for consideration of their possible toxicity in pharmacological and environmental evaluations.

*A. maculatum* is a botanically and pharmacologically important plant species mostly due to its different secondary metabolite profile. Although these phytochemicals show great therapeutic possibilities, their strong toxicological effects call for careful handling and extensive research in any intended use.

## *7.Arum maculatum*: Biochemical Mechanisms of Action

Native to many parts of Europe and Western Asia, *A. maculatum* L., a poisonous member of the Araceae family, assessing its toxic effects following either therapeutic use or accidental ingestion depends on an awareness of its biochemical pathways. Most importantly calcium oxalate crystals, lectins, saponins, and protease inhibitors-that interact with enzymatic activity and cellular architecture to cause cytotoxicity-that plant comprises several biologically active compounds.

### 7.1. Effects at the Level of Cells

Embedded within plant tissues, the calcium oxalate crystals in *A. maculatum* seem as sharp, needle-like formations called raphides. Particularly in the oral and gastrointestinal areas, these structures cause mechanical damage to mucosal membranes that often results in pain, inflammation, and swelling (Franceschi & Nakata, 2005). Beyond physical pain, these crystals might also interact with cell membranes, binding to phospholipids and upsetting normal ion flow across the membrane (Santos et al., 2012).

Furthermore, in this species the lectins bind specifically to surface glycoproteins on epithelial cells. Particularly in the intestinal lining, this interaction can set off cellular absorption mechanisms and aggregation, so upsetting nutrient flow and activating dead pathways (Peumans & Van Damme, 1995). Such lectin-induced effects can disrupt intracellular communication and destabilize cytoskeletal structures, so generating programmed cell death.

### 7.2. Enzymatic disturbance

Essential for protein digestion, trypsin and chymotrypsin are two enzymes whose suppression is well-known from protease inhibitors present in *A. maculatum*. These inhibitors can disrupt appropriate digestion and lead to nutritional deficits and metabolic dysregulation by reducing pancreatic enzyme activity (Valueva & Mosolov, 2004). Moreover, some of

the secondary metabolites of the plant-such as particular alkaloids and flavonoids-are thought to influence cytochrome P450 enzymes, which are fundamental in detoxification pathways (García-Sosa et al., 2006). Another class of poison in *A. maculatum*, saponins show surfactant-like action. These molecules create complexes with membrane cholesterol, so enhancing permeability and allowing pore creation in cell membranes. This action can cause ion leakage and cell rupture (Francis et al., 2002), and hemolysis should red blood cells be impacted.

*A. maculatum*'s poisonous qualities come from their combined and synergistic effects as much as from any one molecule. Structural damage to membranes, inhibition of key enzymes, and disturbance of intracellular signaling comprise the plant's biochemical effect. These interactions highlight the great toxicological risk connected to contact with this plant and the need to know its complicated mode of action at the molecular level.

## 8. Human Health Affects and Toxicological Evaluation

*A. maculatum* is full of various harmful compounds deserving of careful toxicological investigation. Particularly concentrated in the underground structures (rhizomes) and leaves of the plant, saponins, calcium oxalate crystals, and various alkaloids among them considerably influence their toxicity potential (Akbulut & Özkan, 2020). Though they are naturally occurring defenses against herbivores and pathogens for the plant, several compounds can have detrimental effects on people, either ingested or by skin contact.

### 8.1.Toxicity Mechanisms

Among the main toxins in *A. maculatum* are needle-shaped calcium oxalate crystals, known as raphides. These crystals cause mechanical damage, acute inflammation, swelling, and severe discomfort when they touch epithelial tissues, especially on mucosal surfaces. Additionally, when they combine with proteolytic enzymes, they increase corrosive effects, particularly within the gastrointestinal lining (Konno et al., 2021).

By combining into cell membranes, saponins cause cytotoxicity by encouraging pore development and resulting in cellular rupture and disturbed absorption mechanisms. Furthermore, the alkaloids and phenolic compounds of the plant could control neural activity, so affecting the central and peripheral nervous systems either excitatory or inhibitory effect.

### 8.2.$LD_{50}$ Values

Oral $LD_{50}$ for *A. maculatum* extracts shows in rodent models between 800 and 1200 mg/kg in mice (Al-Ali et al., 2008) according to toxicity

testing. One should be aware that solvents used during preparation, dosage forms, and extraction techniques affect such values. Human reports of harmful reactions from consuming raw rhizome material as little as 1–2 grams abound.

### 8.3.Clinical Expressions

Usually starting soon after exposure, *A. maculatum* poisoning symptoms include a sharp burning sensation in the mouth, throat, and esophagus. Usually, this is followed by dyspnea, nausea, vomiting, dyspagia, and stomach cramps. Excessive salivation is also common (Pietrzak et al., 2016). In more severe instances, swelling of the larynx can lead to breathing difficulties or respiratory distress. Skin exposure may provoke localized reactions, such as itching, redness, and contact dermatitis.

### 8.4.Possible Antidotes and Treatment

Although there is no specific antidote, treatment is symptomatic and supportive. In case of oral exposure, the toxic substance can be diluted by drinking milk or water. In some cases, activated charcoal may be useful. In case of respiratory tract involvement, airway safety is the priority. Antihistamines and corticosteroids can be used to control edema and inflammation. In most patients, symptoms regress within 24-48 hours; however, serious complications may develop in some sensitive individuals. The toxic effects of the plant are not known among the public and pose a serious risk, especially for children and the elderly. Therefore, it is of great importance to conduct informative studies in terms of public health and to prevent accidental consumption of the plant (Gündoğdu & Özsoy, 2021).

## 9.Potential Pharmacological Effects and Research Directions of *Arum maculatum*

*A. maculatum* is a species that has traditional use among medicinal plants but has recently begun to be studied in terms of modern pharmacology. Studies on its antimicrobial, anticancer and anti-inflammatory effects in particular reveal the biological activity potential of the plant. *In vitro* studies on the antimicrobial effects of the plant show that especially leaf and rhizome extracts are effective against various bacterial and fungal species. In analyses using methanol, ethanol and water-based extracts, significant inhibitory effects on common pathogenic microorganisms such as Staphylococcus aureus, Escherichia coli, Candida albicans were observed (Sönmez et al., 2006). These effects are thought to be related to the presence of flavonoids, phenolic compounds and some alkaloids.

The anticancer potential of *A. maculatum* has been addressed in a limited number of books. However, the initial polyphenol-rich composition and free radical scavenging capacity provide clues that cytotoxic effects may be produced in the cancer system. In particular, rhizome extracts have been shown to have apoptotic and anti proliferative effects in some human cancer cell lines (Kantidze et al., 2019). However, the nature of these effects and dose-dependent responses have not yet been sufficiently elucidated. Therefore, comprehensive in vivo and clinical studies are needed. In terms of anti-inflammatory effects, *Arum maculatum* has long been known to be used against rheumatic disorders. Modern experimental studies suggest that the plant contains some components that act as cyclooxygenase (COX) inhibitors (Yılmaz et al., 2021).

*A. maculatum* is hypothesised to down regulate nitric oxide generation, so having anti-inflammatory action. More validation using in vivo animal models and in vitro cell culture systems is needed, though, to support these first findings. Although the plant exhibits possible pharmacological effects, current studies insufficiently support clinical use. Any therapeutic use has to carefully consider the benefit-risk ratio given the known toxicological hazards connected to its bioactive components. Next research should mostly focus on development of standardized plant extracts, isolation and characterization of bioactive molecules, and identification of their molecular mechanisms of action.

## 10.Identification and Quantification of Bioactive Constituents: Chemical Analysis Methods

Extensive chemical profiling is necessary for precise definition of the pharmacological and toxicological aspects of *A. maculatum*. This suggests the use among advanced analytical methods high-performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FT-IR), and gas chromatography-mass spectrometry (GC-MS). These techniques let one evaluate the metabolites of the plant both qualitatively and quantitatively together with possible links with biological activity (Ali et al., 2024).

Particularly sensitive, GC-MS helps one locate and characterize volatile and semi-volatile molecules. In a 2017 Rahman et al. paper, 24 unique bioactive compounds were found by GC-MS analysis of methanolic extracts of *A. maculatum* rhizomes. Most commonly occurring among these are phytol (10.3%), palmitic acid (hexadecanoic acid, 18.4%), and oleic acid (9-octadecenoic acid, 22.6%). The antimicrobial and anti-inflammatory properties of these substances fit the historical use of the plant in traditional medicine.

Many times, HPLC is used to separate and measure phenolic compounds and flavonoids. Using HPLC to examine *A. maculatum* leaf and stem extracts, Mizzi et al., (2020) identified compounds including rutin (1.63 ± 0.11 mg/g), caffeine (3.25 ± 0.12 mg/g), and chlorogenic acid (2.47 ± 0.09 mg/g). Since they can neutralize reactive oxygen species, these polyphenols are particularly amazing in terms of their antioxidant power and possible cardioprotective action. Especially, the environmental surroundings and extraction techniques determine the concentration of these phenolic elements.

One qualitative tool for functional group identification in plant extracts is FT-IR spectroscopy. Important absorptions between 3300–3400 $cm^{-1}$, C–H stretching at 2920 $cm^{-1}$, and carbonyl (C=O) signals near 1740 $cm^{-1}$ were reported by Demir et al. (2015) in FT-IR spectra of *A. maculatum* rhizome extracts. These spectral traits imply ester functions, lipid moieties, and phenolic structures. Emphasizing the consistency of a multi-method approach, the FT-IR results fit rather nicely with data acquired by GC-MS and HPLC.

Combining several analytical methods helps to holistically assess the phytochemical composition of *A. maculatum*. Moreover, strongly influenced by elements such as solvent type (e.g., methanol, ethanol, hexane), extraction length, and temperature are the efficiency and specificity of compound extraction. If one wants chemically accurate and repeatable profiles, these values have to be carefully adjusted.

Chemical analysis of *A. maculatum* discloses a complex array of biologically active substances awaiting interest from therapeutic along with toxicological perspectives. The abundance of lipid-derived substances such as oleic and palmitic acids in *A. maculatum* indicates a probable anti-inflammatory activity. However, the occurrence of certain cytotoxic substances cautions for prudent interpretation and extensive safety evaluation (Rahman et al., 2017).

**Conclusions**

*A. maculatum* is both a toxic and medicinal plant. Though traditional medicine has used it for centuries, modern science is just starting to uncover precisely how. Some research has indicated that its extracts may combat infection, decrease inflammation, or even treat cancer. Yet, it also has toxic elements that, if used incorrectly, can lead to severe medical issues. This is interesting but also perilous. Further study is necessary, particularly study of its effects on the human body in real-life applications. *A. maculatum* could provide new possibilities for natural medicine with correct application and in sufficient dosage. But without knowledge, it can

cause more harm than benefit. Therefore, before utilizing this plant in the health sector, scientific research, public awareness, and safety guidelines are all important duties.

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# Chapter 7

# MEDICAL USE OF SOME IMMOBILIZED PANCREATIC ENZYMES

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## INTRODUCTION

Enzymes function as biocatalysts, accelerating biological reactions with high specificity. Their remarkable catalytic efficiency, specificity, and biocompatibility render them broadly applicable across industrial and medical sectors. However, challenges inherent in using free enzymes include their limited stability, inability to be reused, difficulties in achieving product purity, and concerns regarding economic sustainability. To address these issues, enzyme immobilization (enzyme fixation) has been developed as a technique that involves binding enzymes to a specific surface or matrix, thereby preventing their dissociation from solution (Datta et al., 2013; Sheldon, 2007).

Enzyme immobilization is accomplished by either physically or chemically attaching the enzyme to a support material or by physically entrapping it within a matrix. Common immobilization techniques encompass adsorption, covalent binding, cross-linking, entrapment, and encapsulation (Mateo et al., 2007). Support materials can range from natural polymers (e.g., alginate, agarose) to synthetic polymers (e.g., polyacrylamide), as well as inorganic surfaces (e.g., silica, glass beads) and nanoparticles (Mohamad et al., 2015). These methods enhance enzyme resilience to environmental stressors while simultaneously enabling their reuse.

The use of immobilized enzymes in the medical field provides significant advantages especially in diagnosis, treatment and drug development processes. Most of the biosensors used in diagnostic tests contain immobilized enzymes. For example, glucose oxidase enzyme is immobilized and used in glucose biosensors, which are widely used to determine glucose levels (Wang, 2008). In addition, enzymes such as urease, choline oxidase and lactate oxidase are also used in various biosensor applications (Turner, 2013).

In addition, some enzymes are used directly for therapeutic purposes. For example, asparaginase enzyme is used especially in the treatment of acute lymphoblastic leukemia and its immunogenicity is reduced and its half-life in circulation is extended by PEG (polyethylene glycol) modification (PEGylation) and immobilization (Veronese & Mero, 2008). Lactase enzyme is presented in an immobilized form in supplementary products used for individuals with lactose intolerance. Similarly, antioxidant enzymes such as catalase and superoxide dismutase are used in medical formulations to protect against free radical damage (Zimecki & Artym, 2005).

In recent years, thanks to the developments in nanotechnology, the use of nanoparticle-based supports for enzyme immobilization has accelerated. These developments further increase enzyme stability and enable the

development of targeted transport systems (Li et al., 2019). In addition, immobilized enzymes integrated with microfluidic systems and lab-on-a-chip technologies offer rapid and sensitive diagnosis with minimal sample (Sia & Kricka, 2008).

Systems obtained by enzyme immobilization not only increase the efficiency of biochemical reactions but also offer significant advantages in terms of cost-effectiveness and sustainability. The development of immobilized enzymes used in medicine and their dissemination in clinical applications paves the way for revolutionary advances in both diagnosis and treatment. In this study, enzyme immobilization techniques and current applications of immobilized enzymes used in medicine will be examined in detail (Layer & Keller, 2003).

Pancreatic enzymes are enzymes that perform digestive functions such as amylase, lipase and protease and are used as external supplements, especially in the treatment of conditions such as exocrine pancreatic insufficiency (EPI). Classical forms of these enzymes have disadvantages such as being unstable to stomach acid and having reduced activity in the small intestine. Therefore, immobilization of pancreatic enzymes stands out as an effective strategy to increase stability in the gastrointestinal environment and maintain therapeutic efficacy. Pancreatic enzyme preparations, especially those immobilized on enteric-coated microgranules, dissolve in the targeted area (duodenum and jejunum) and provide optimum digestive support. This technology is an important treatment approach, especially in cystic fibrosis, chronic pancreatitis and digestive disorders that develop after pancreatectomy (Layer & Keller, 2003; Thorat et al., 2012; Waljee et al., 2009). In addition, immobilized pancreatic enzymes have started to find a place in biotechnological diagnostic platforms in recent years. These enzymes are used in a immobilized form in biosensor systems that allow rapid diagnosis of disorders related to fat digestion, for example, and are helpful in clinical diagnosis. In addition, stable and controlled release formulations of these enzymes can be combined with oral drug delivery systems to contribute to individualized treatment. Pancreatic enzymes immobilized on nanotechnology-based support surfaces (e.g., silica nanoparticles or polymeric microcapsules) increase clinical success by both preserving enzymatic activity and providing controlled distribution in the gastrointestinal tract (Jia et al., 2022; Zhao et al., 2022).

**Amylases**

Alpha-amylase (EC 3.2.1.1) represents an endo-amylase that specifically breaks down complex polysaccharides such as starch and glycogen. This enzyme cleaves glucose units along α-1,4-glycosidic bonds to con-

vert them into smaller sugars such as maltose, maltose, and dextrin (Van Der Maarel et al., 2002). In humans, alpha-amylase exists in two main forms: salivary alpha-amylase (AMY1) and pancreatic alpha-amylase (AMY2). Starch hydrolysis begins in the saliva and continues in the small intestine with amylase produced by the pancreas (Swensson et al., 1985). The digested starch products exist as then absorbed from intestinal cells and used for energy production (Ramasubbu et al., 1996).

Amylase enzyme was immobilized by different methods: immobilization of amidrazone onto acrylic fabric by covalent bonds enhanced the thermal stability and pH tolerance of the enzyme (Al-Najada et al., 2019). Immobilization of α-amylase from Aspergillus fumigatus onto chitin-bentonite hybrid matrix by adsorption enhanced the stability of the enzyme (Tiarsa et al., 2022). Immobilization of the enzyme by cross-linking in gellant particles provided protection against environmental factors and enhanced its reusability. Immobilization on ZIF-8 nanocomposite strengthened the performance of the enzyme (Atiroğlu et al., 2021; Tincu et al., 2023).

**Lipases**

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) hydrolyse triglycerides to diglycerides, monoglycerides, free fatty acids, and glycerol (Anobom et al., 2014). Lipases play a critical role in the digestion and metabolism of fats; pancreatic lipase, in particular, converts dietary triglycerides to monoacylglycerols and free fatty acids, facilitating their absorption in the small intestine (Winkler et al., 1990). A comprehensive review was conducted on the use of immobilized lipases in the biocatalytic synthesis of short-chain esters. The study indicated that lipases provide high efficiency and selectivity in esterification reactions, but short-chain acid and alcohol substrates can inhibit enzyme activity (Sousa et al., 2025).

Lipase enzyme was immobilized by covalently binding to $Fe_3O_4$ magnetic nanoparticles. The obtained biocatalyst was successfully used in the production of biodiesel from waste cooking oil. The enzyme could be reused 10 times and provided over 90% efficiency (Tong et al., 2021). Lipase was immobilized on support materials developed using 3D printers with PLA (Polylactic Acid) filaments. With this method, customizable biocatalyst surfaces were designed (dos Santos et al., 2021). Lipase enzyme was entrapped in gel beads obtained using sodium alginate and calcium chloride. This system underwent evaluation to control lipolysis reactions in cheese production (Chakraborty, 2017). Immobilized lipase also has applications in the health field. They evaluated the efficacy of an in-line immobilized lipase cartridge aimed at improving fat absorption during

tube feeding in cystic fibrosis (CF) patients. CF patients have difficulty digesting fats due to pancreatic insufficiency. The study found that this cartridge breaks down fats in the nutritional formula before they reach the patient, allowing them to be better absorbed. The results demonstrated that the cartridge significantly enhanced fat absorption and could improve the nutritional status of patients. This technology offers a new and effective solution, especially for CF patients in whom oral enzyme therapy represents inadequate (Sathe et al., 2021). They examined the management of exocrine pancreatic insufficiency (EPI), which represents common in patients with pancreatic adenocarcinoma. They evaluated the efficacy of immobilized lipase cartridges used in oral pancreatic enzyme therapy (PERT) and enteral feeding in a group of 35 patients. EPI symptoms were seen in 80% of the patients and the symptoms decreased with treatment. Early EPI management was found to be important in improving quality of life and facilitating return to oncological treatment (Moore et al., 2024).

**Trypsin**

Trypsin (EC 3.4.21.4) represents among the serine endopeptidases and hydrolyses peptide bonds, breaking down proteins into smaller peptides. These peptide products exist as then hydrolysed by other proteases to amino acids, allowing their absorption into the blood (Li et al., 2007). Trypsin obtained from porcine pancreas was immobilized onto glutaraldehyde-activated chitosan hydrogels. The immobilized enzyme exhibited high catalytic performance in the hydrolysis of BSA and was stable under different pH conditions. It also retained its activity in reusability tests, demonstrating suitability for industrial applications (Miguez et al., 2023).

Trypsin was immobilized on programmable DNA tetrahedrons. This structure enhanced protein digestion efficiency by controlling the distribution and orientation of trypsin on the surface. In addition, immobilized trypsin provided faster and more efficient digestion than traditional methods (Urzúa et al., 2022). Trypsin was covalently immobilized on the surface of porous methacrylate-based monoliths. The immobilized enzyme demonstrated high efficiency in protein digestion and was stable under various pH and temperature conditions. It also retained its activity in reusability tests, demonstrating suitability for industrial applications (Rodzik et al., 2023). Digestion of milk proteins was performed using immobilized trypsin enzyme microreactors (μ-IMER). The μ-IMER method provided faster and more efficient digestion compared to the traditional in-gel digestion method. In addition, immobilized trypsin offered advantages in terms of reusability and stability. Trypsin enzyme was immobilized on natural and synthetic polymer hydrogels containing magnetite ($Fe_3O_4$) nanoparticles. Magnetite nanoparticles were integrated into the

hydrogel matrix by co-precipitation method. This structure enhanced the immobilization efficiency and biological activity of the enzyme. In addition, magnetite-containing hydrogels significantly strengthened the stability and specific activity of the enzyme. The results demonstrated that the presence of magnetite nanoparticles enhanced the immobilization degree and biological activity of the enzyme (Sun et al., 2014).

In the study titled "Uncovering Immobilized Trypsin Digestion Features from Large-Scale Proteome Data Generated by High-Resolution Mass Spectrometry", it was observed that immobilized trypsin digested proteins approximately twice as fast as free trypsin in solution. Protein identifications and proteome depth achieved using immobilized trypsin were found to be similar or comparable to those obtained with solution-based trypsin digestion. One of the most striking findings was that a short digestion time of only 5 minutes with immobilized trypsin resulted in a comparable number of missed cleavages to those generated by a 4-hour digestion using solution-phase trypsin. This indicates that immobilized trypsin provides a significantly faster and more efficient digestion process. Additionally, the study reported that immobilized trypsin enabled quantitatively reproducible digestion of complex proteomes, with a small but measurable loss of peptides due to adsorption (Rawlings et al., 2018; Sun et al., 2014).

**Carboxypeptidase**

Carboxypeptidases (EC 3.4.17.) exist as exopeptidase enzymes that hydrolyze and remove single amino acids from the C-terminus (carboxyl end) of proteins and peptides. These enzymes exist as included in group EC 3.4.17 according to the Enzyme Commission (EC) classification and belong to the metalloprotease (zinc-dependent) or serine protease subclasses (Ganie et al., 2021). Carboxypeptidase A covalently bound to $Fe_3O_4$ magnetic nanoparticles demonstrated high activity in peptide hydrolysis reactions. The operational stability of the enzyme was significantly enhanced, and it was observed that it retained 85% of its initial activity even after 10 cycles of use. The easy recovery of the enzyme by magnetic separation provided advantages in industrial applications. The study revealed that immobilization strengthened the pH and heat stability of the enzyme (Garg et al., 2015).Carboxypeptidase E immobilized on the graphene oxide surface enhanced the neuropeptide processing rate by 2.5 times compared to the free enzyme. The enzyme loading capacity was significantly enhanced due to the high surface area. The biocatalyst retained 70% of its initial activity even after 15 cycles of use. The study demonstrated that this system can be used in the investigation of neuroendocrine disorders (Křenková & Foret, 2004).

Carboxypeptidase B immobilized in the microfluidic chip produced peptides in high yield in a continuous flow system. The system provided 5-fold higher product yield than conventional batch reactors. The enzyme retained 80% of its activity after 30 days of continuous operation. The study demonstrated that this method offers a scalable approach for pharmaceutical peptide production (Südi et al., 1989). CPA and CPB immobilized by cross-linked enzyme aggregates (CLEA) method demonstrated synergistic effect in industrial protein hydrolysis. Co-immobilization of two enzymes provided 40% higher yield compared to single systems. The biocatalyst proved its suitability for industrial processes by remaining stable at 50°C for 24 h. The study emphasized that this method provides a cost-effective industrial process (Bertino et al., 1978).

The study titled "Immobilized Carboxypeptidase G1 in Methotrexate Removal" investigated the immobilized form of the enzyme Carboxypeptidase G1 (CPG1), which plays a critical role in the treatment of methotrexate (MTX) poisoning. CPG1 represents an enzyme that can convert methotrexate and other folate analogs into inactive metabolites. The article investigated the efficacy and stability of CPG1 immobilized on nylon tubes and hollow fibers for use in extracorporeal enzyme reactors (external enzyme reactors). They found that immobilized CPG1 had high stability on nylon tubes and hollow fibers, and that the reactor parameters supported the enzyme's methotrexate removal capacity at flow rates and methotrexate concentrations of clinical interest. In vivo experiments in dogs clearly demonstrated that such extracorporeal shunts have potential applications in "rescue" therapy after high-dose MTX administration. For dogs, approximately It was stated that the CPG1 reactor with a cleaning value of 150 ml would be required for such applications (Bertino et al., 1978).

## CONCLUSION

Enzyme immobilization techniques offer significant advantages over free enzymes in terms of increased stability, reusability, and ease of product purification and economic sustainability. These methods involve binding enzymes to various support materials like natural or synthetic polymers, inorganic surfaces, and nanoparticles. The application of immobilized enzymes in the medical field provides significant benefits, particularly in diagnosis, treatment, and drug development. They are widely used in biosensors for detecting substances like glucose, urea, choline, and lactate. Furthermore, immobilized enzymes such as asparaginase and lactase are directly employed for therapeutic purposes, improving efficacy and stability in biological systems. Recent advancements in nanotechnology have further enhanced the use of nanoparticle-based supports,

leading to improved enzyme stability and the development of targeted delivery systems.

Pancreatic enzymes, including amylase, lipase, and protease, are crucial for digestive functions and are used as external supplements, especially in treating exocrine pancreatic insufficiency (EPI). Immobilization of these enzymes effectively addresses issues like instability in stomach acid and reduced activity in the small intestine, thus enhancing therapeutic efficacy. This approach is particularly beneficial for conditions such as cystic fibrosis, chronic pancreatitis, and post-pancreatectomy digestive disorders, where enteric-coated, immobilized preparations ensure optimal digestive support in the targeted areas of the duodenum and jejunum. Beyond therapeutic applications, immobilized pancreatic enzymes are also being utilized in biotechnological diagnostic platforms for rapid diagnosis of fat digestion disorders. Nanotechnology-based supports further improve their clinical success by preserving enzymatic activity and enabling controlled distribution within the gastrointestinal tract.

Specifically, the study on immobilizing porcine trypsin in superparamagnetic nanoparticles demonstrated that this method significantly improves enzyme activity and stability, enabling faster and more efficient protein digestion for large-scale proteome analysis compared to traditional methods. Similarly, the research on immobilizing Carboxypeptidase G1 (CPG1) showed its high effectiveness and stability in extracorporeal enzyme reactors for rapid methotrexate removal in patients with high-dose MTX poisoning. These findings underscore the transformative potential of immobilized enzymes in revolutionizing both diagnostic and therapeutic strategies in medicine.

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# Chapter 8

# THE HETEROCYCLIC WORLD OF PHARMACEUTICALS

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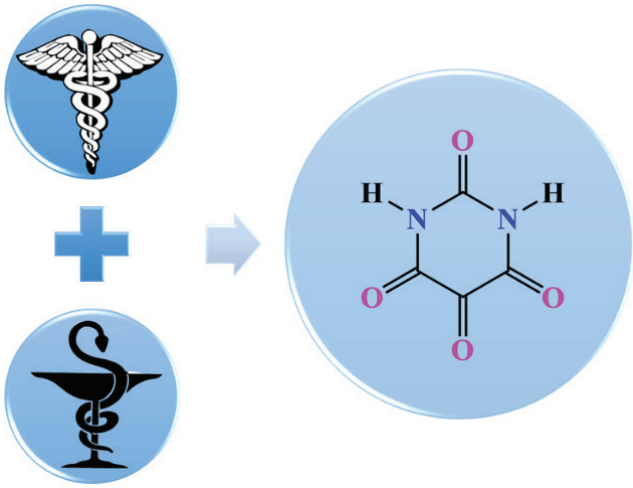
Medicinal chemistry emerged as a discipline through the integration of knowledge derived primarily from organic chemistry, along with insights from pharmacy, biology, and general chemistry. Initially, natural therapeutic compounds used in the diagnosis and treatment of diseases were obtained by purification from plant and animal tissues or through microbial fermentation. However, later advancements in organic chemistry enabled the laboratory synthesis of these compounds (Slman et al., 2024). The intriguing pharmacological properties of organic molecules have significantly contributed to the growing prevalence of synthetic drug design in modern medicine. Due to the exceptional bonding capacity of the carbon atom which is considered the fundamental element of organic chemistry, and the structural framework provided by covalent C–H bonds, a wide variety of structurally diverse compounds can be synthesized. A systematic approach is employed to examine the structures, synthesis, characteristic properties, and reactions of these compounds, enabling the classification of complex organic structures (Mallappa et al., 2025).



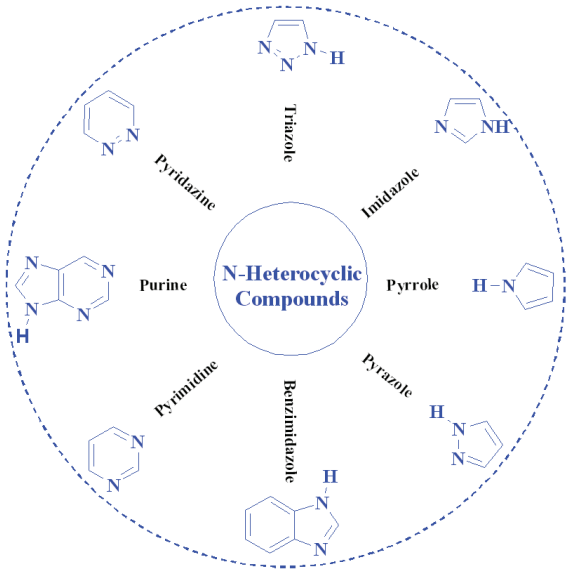

Cyclic structures, a fundamental component of organic systematics, refer to ring-shaped molecular frameworks formed by the covalent bonding of atoms in a closed loop. The substitution of one or more carbon atoms in the cyclic framework with heteroatoms such as nitrogen (N), oxygen (O), sulfur (S), or phosphorus (P) results in the formation of monocyclic or polycyclic heterocyclic compounds (HCs) (Jha et al., 2023; Qadir et al., 2022). Because all natural or synthetic HCs exhibit physiological and pharmacological effects, investigating these unique structures has become a key focus in contemporary research (Jha et al., 2023; Qadir

et al., 2022). These aromatic and aliphatic ring systems are referred to as HCs in synthetic chemistry. Regarded as dating back to the origins of organic chemistry, the first known heterocycle is **alloxan**, a pyrimidine derivative, synthesized by chemist Brugnatelli via the oxidation of uric acid with nitric acid (Kumar et al., 2023; Slman et al., 2024). The presence of heterocyclic frameworks in endogenous substances of living organisms, as well as in phytochemicals with bioactive properties such as flavonoids, alkaloids, carotenoids, and phenolic acids, further underscores the growing significance of heterocyclic systems in modern drug design (Rizzo et al., 2023).

HCs are considered ideal candidates for biological pathways and enzyme binding sites due to their hydrogen bond donor/acceptor activities, ability to form coordinate covalent bonds, exhibit π-bond reactivity or delocalization, engage in weak intermolecular interactions, and the potential for structural modification of the ring system (Jha et al., 2023). Today, many drugs approved by the Food and Drug Administration (FDA) contain aromatic heterocyclic compounds (HCs) in their structures (Luo et al., 2024). The presence of nitrogen, sulfur, oxygen, and fluorine atoms in the core ring alters the physicochemical properties of HCs, which in turn affects their therapeutic profiles (Kumar et al., 2023).

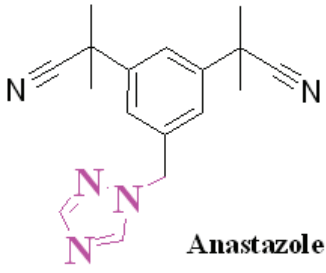
**Nitrogenous Heterocyclic Compounds and Their Biological Activities**

The pharmacological effects of nitrogen-containing rings found in biological compounds such as deoxyribonucleic acid (DNA), vitamins, coenzymes, and hormones have contributed to the widespread use of these structures as active cores in drug design (Kumar et al., 2023; Mallappa et al., 2025). Nitrogen-containing heterocyclic compounds (NCHCs) and their ring analogs represent a crucial class in pharmaceutical chemistry, owing to their synthetic versatility and structural adaptability for drug development. Their advantages such as low toxicity, high bioavailability and biocompatibility, minimal drug resistance, and favorable reaction conditions make these compounds excellent candidates for synthetic drug discovery (Mallappa et al., 2025). Today, heterocyclic groups containing nitrogen atoms in their structural frameworks, such as triazole, imidazole, pyrrole, pyrazole, benzimidazole, pyrimidine, and pyridazine, constitute one of the most significant classes of pharmaceutical agents.



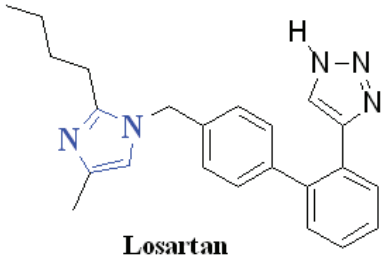

## Triazole

Triazole is a five-membered heterocyclic compound containing three nitrogen atoms in its ring structure. Due to their bioactive properties, both 1,2,3-triazole and 1,2,4-triazole derivatives have been extensively studied for therapeutic applications (Kumar et al., 2023). Triazole derivatives exhibit a wide range of pharmacological activities, including antimicrobial, analgesic, anti-inflammatory, local anesthetic, anticonvulsant, antimalarial, anti-HIV, antidiabetic, hypoglycemic, anxiolytic, antihypertensive, and antioxidant effects (Gharge & Alegaon, 2024). Moreover, because of their ability to inhibit cell division, prevent metastasis, and activate apoptosis, they are considered promising candidates for cancer therapy. **Anastrozole**, which possesses a triazole core, is one of the well-known active pharmaceutical agents (Mallappa et al., 2025).



## Imidazole

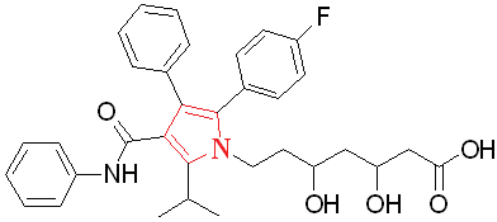
Imidazole is a heteropentacyclic compound characterized by a five-membered aromatic ring containing two nitrogen atoms. This molecule, which constitutes the core structure of many bioactive agents in pharmacology, has a high dipole moment, making it soluble in polar solvents (Mohammed et al., 2023). The imidazole ring, found in the structure of histidine, a key amino acid involved in DNA transcription, is one of the indispensable scaffolds in drug synthesis (Kumar et al., 2023). The pharmacological mechanisms of imidazole derivatives include the suppression of pro-inflammatory cytokines and enzymes, optimization of insulin sensitivity, regulation of vascular resistance and blood pressure, and interference with viral replication. **Losartan**, which contains an imidazole ring, is among the well-known active pharmaceutical agents (Mallappa et al., 2025).




**Losartan**

## Pyrrole

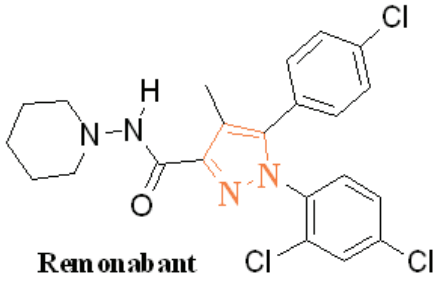
The pyrrole ring and its derivatives, found in biological molecules such as chlorophyll, vitamin B12, and bile pigments like heme, represent important class of organic HCs. Numerous therapeutics containing a pyrrole core exhibit antimicrobial, antioxidant, antiviral, antibacterial, anti-inflammatory, and antifungal activities. Recent studies have reported that drug structures incorporating pyrrole rings and substituted pyrroles not only possess anticancer properties but also demonstrate specific growth-inhibitory activity against human cancer cell lines, including those associated with breast cancer and chronic myeloid leukemia. Atorvastatin, which contains a pyrrole ring, is a pharmaceutically active agent (Jha et al., 2023).




**Atrovastatin**

***Pyrazole***

Pyrazole is an aromatic heterocyclic compound characterized by a five-membered ring containing two adjacent nitrogen atoms. The presence of nitrogen atoms acting as both electron donors and acceptors facilitates interactions with a wide range of enzymes and receptors, which is a key factor underlying the pharmacological activity of pyrazole. Synthetic derivatives bearing a pyrazole core are known to exhibit antibacterial, antiviral, anti-inflammatory, and cyclooxygenase (COX) inhibitory effects. Furthermore, their biological activity in enzyme-receptor interactions that drive cancer cell proliferation supports their potential use as anticancer agents (Al-Jumaili et al., 2023).

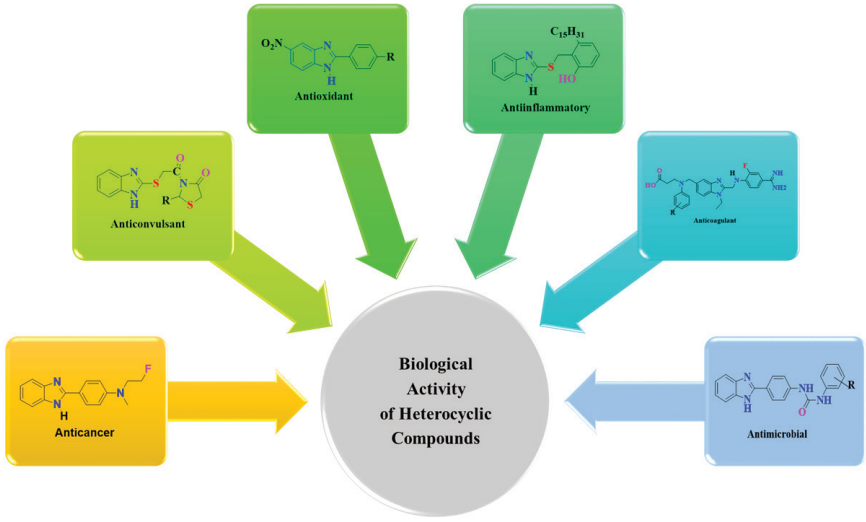



**Rimonabant**

In addition, their neuroprotective properties and ability to inhibit enzymes such as angiotensin-converting enzyme (ACE) and carbonic anhydrase have made them subjects of extensive research in drug development. Beyond their applications in medicine, pyrazoles are also used in the chemical industry as UV stabilizers, cosmetic and food colorants, and agrochemicals (Kumar et al., 2023).

## Benzimidazole

Benzimidazole, formed by the fusion of benzene and imidazole rings, is an aromatic and planar HCs. Its perfect coordination with the central cobalt ion in vitamin B12 and the structural integrity provided by the benzimidazole core have guided researchers in exploring its therapeutic applications. The benzimidazole ring and its analogs exhibit activity against hypotension (Mohammed et al., 2023), ulcers, histamine-related conditions, hypertension, bacterial infections, and inflammation. Moreover, the incorporation of amino or amido groups into the side chains of the benzimidazole structure has been reported to result in antiproliferative activity against human cancer cell lines, demonstrating high affinity as potential anticancer agents (Kumar et al., 2023).



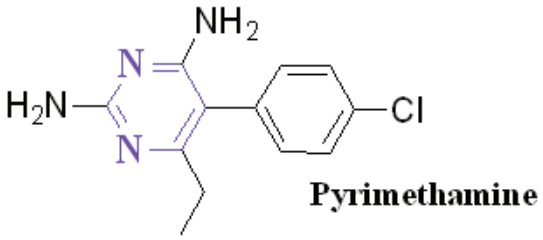

## Pyrimidine

Pyrimidine is a stable six-membered aromatic HCs, structurally analogous to benzene and characterized by the presence of two nitrogen atoms at the 1,3-positions. Its natural occurrence in fundamental biomolecules such as DNA and RNA necessitates the investigation of its biological activity in modern medicine (Jha et al., 2023). Various modified pyrimidine rings exhibit pharmacological properties including anticonvulsant, antihypertensive, antimicrobial, antiviral, anti-inflammatory, and muscarinic agonist effects. Notably, the tetrahydropyrimidine ring plays a critical role in DNA–protein interactions by contributing to thermal stability and salt sensitivity. Pyrimidine nucleoside analogs, which are among the

most important pharmaceutical compounds, show strong pharmacological potential against herpes viruses and HIV by directly inhibiting DNA or RNA synthesis during viral replication.

Specific pyrimidine side-chain derivatives also interfere with DNA synthesis in cancer cells, thereby slowing tumor progression and disease development, and are utilized in the treatment of pancreatic cancer and myeloid leukemia (Kumar et al., 2023; Mushtaq et al., 2024).

**Pyrimethamine**, a pyrimidine derivative, selectively inhibits the dihydrofolate reductase (DHFR) enzyme essential for the life cycle of Plasmodium falciparum the parasite responsible for malaria without affecting human DHFR, making it a selectively toxic therapeutic agent (Mallappa et al., 2025).

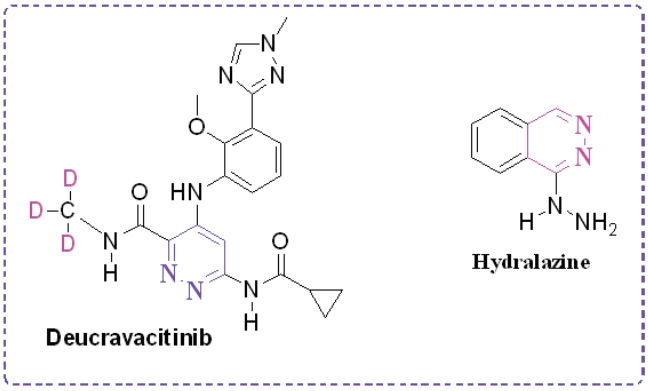



**Pyrimethamine**

**Pyridazine**

Pyridazine is a heterocyclic diazine compound characterized by a six-membered ring containing two adjacent nitrogen atoms at the 1,2-positions. The physicochemical properties believed to underlie the pharmacological effects of the pyridazine ring help explain its potential in drug discovery (Al-Jumaili et al., 2023). Electron deficiency at the C-3 and C-6 positions is attributed to the presence of substituents and the overall lipophilicity of the ring. Due to its high dipole moment, the structure is amenable to both intramolecular and intermolecular interactions. The unshared electron pairs on the adjacent nitrogen atoms enhance the ring's hydrogen bond acceptor capacity while contributing to its low intramolecular basicity. The functional properties of the pyridazine core such as intramolecular hydrogen bonding facilitate membrane permeability, while its conformational alignment, supported by amide groups, is critical for enzyme interactions (Meanwell, 2023). Therapeutic compounds containing the pyridazine scaffold exhibit a wide range of biological activities, including antiplatelet aggregation, analgesic, antidepressant, antiviral, antitubercular, and anticancer effects. Notably, compounds like **hydrala-**

**zine** and cadralazine possess antihypertensive properties, and their structural efficacy has proven beneficial in the treatment of cardiovascular disorders, chronic pain, and inflammatory diseases (Mallappa et al., 2025).

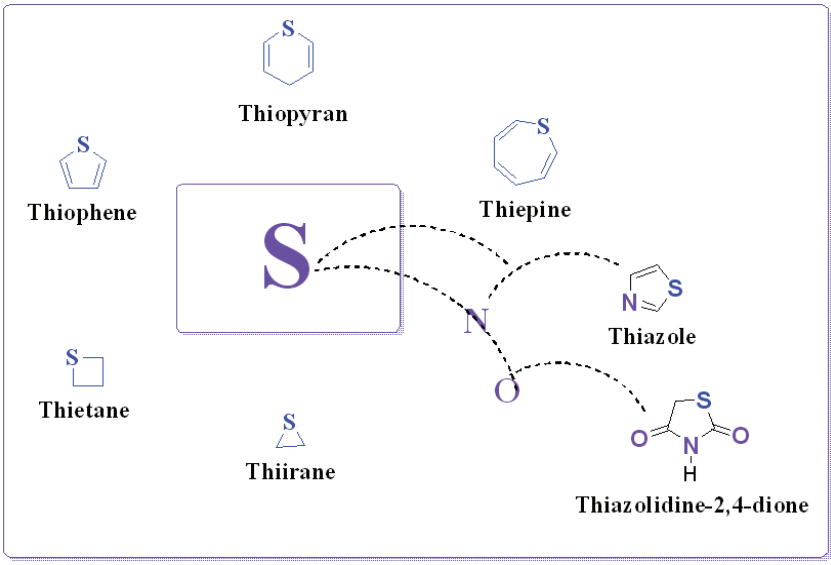



**Deucravacitinib** is the first 'de novo deuterated' drug designed as a therapeutic agent for moderate-to-severe plaque psoriasis (Meanwell, 2023). In addition to their pharmacological applications, pyridazine rings are also key structural components in herbicides within the field of agricultural chemistry.

**Biological Activities of Sulfur-Containing Heterocyclic Compounds**

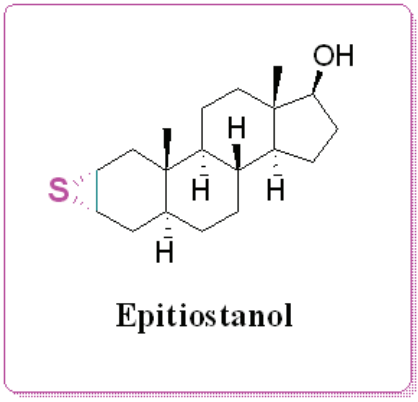
The necessity of sulfur atoms in metabolic processes such as oxidative stress and detoxification, along with the presence of sulfur-containing amino acids like methionine and cysteine, renders sulfur-containing heterocycles highly attractive compounds in pharmaceutical chemistry. The investigation of the chemical structures of sulfur heterocyclic drugs necessitates their classification according to ring size (Kapoor et al., 2025).

The most therapeutically significant sulfur-containing rings are three-, four-, five-, six-, and seven-membered heterocycles. The direct incorporation of sulfur atoms into the active core structure enhances biological activities such as lipophilicity, bioavailability, and metabolic stability, while the presence of secondary and tertiary heteroatoms like nitrogen and oxygen in the same ring confers additional pharmacological advantages in their mechanisms of action (Al-Jumaili et al., 2023). Various sulfur-containing HCs and their heteroatom derivatives such as thiirane, thietane, thiophene, thiopyran, and thiazepine have been demonstrated in studies to possess antimicrobial, antidiabetic, antifungal, antiviral, antibacterial, anticancer, anti-inflammatory, antihypertensive, antimalarial, and anti-Alzheimer activities (Mallappa et al., 2025).



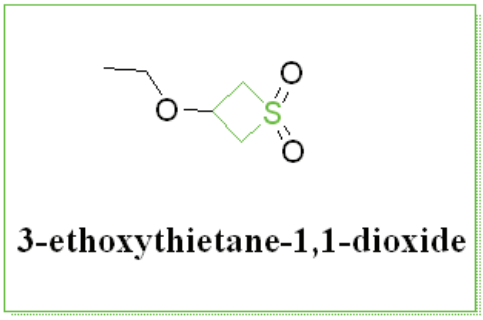

## Thiirane

Ethylene sulfide, a heteromonocyclic compound containing a sulfur atom within a three-membered ring, is characterized by a strained ring structure typical of three-atom heterocycles. This ring strain contributes to the biological activity of drug molecules bearing this core (Kapoor et al., 2025). **Epitiostanol**, designed as an antiestrogenic and antineoplastic agent, is a potent androgenic-anabolic steroid that reduces estrogen levels and inhibits the proliferation of tumor cells, making it effective in the treatment of breast cancer and gynecomastia (Ungureanu et al., 2024).



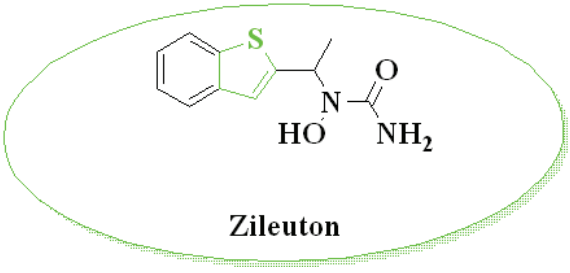
### Thietane

Thietane is a four-membered saturated strained ring molecule containing three carbon atoms and one sulfur atom within its heterocyclic structure. Although some oxidized derivatives exhibit antidepressant and antioxidant properties, thietane remains one of the least studied sulfur-containing heterocyclic compounds. The thietane derivative, **3-ethoxythietane-1,1-dioxide**, has been reported to demonstrate neuropharmacological activities (Ungureanu et al., 2024).



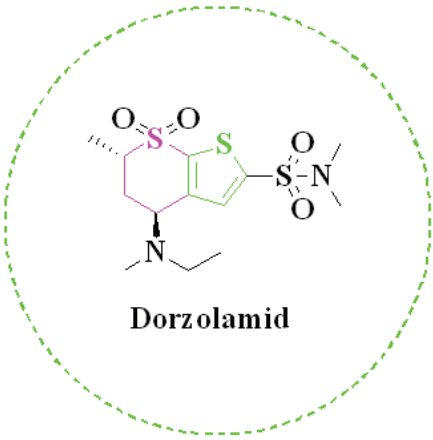

### Thiophene

Benzothiophene is a five-membered aromatic HCs containing a sulfur atom, which has garnered significant interest in medicinal chemistry due to its therapeutic properties. Specific substitutions and structural modifications within the ring enable its application as an antitumor, antitubercular, anti-inflammatory, antioxidant, and anti-HIV agent. **Zileuton**, a leukotriene synthesis inhibitor used in the treatment of asthma and allergic rhinitis, contains the benzothiophene heterocyclic ring (Ungureanu et al., 2024).



**Thiopyran**

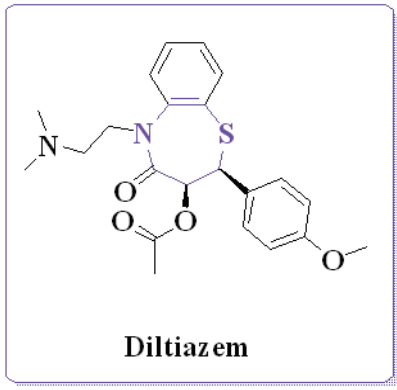
Thiopyran is a compound formed by the replacement of the oxygen atom with sulfur in the six-membered pyran ring, existing as two different isomers depending on the position of the unsaturated π-bond. The pharmaceutical efficacy of oxygen- and nitrogen-containing derivatives of this ring has promoted the use of the thiopyran core as a fundamental scaffold in organic synthesis. Interest in the thiopyran ring is increasing due to its antipsychotic, antiviral, antibacterial, and antihyperplasia activities. **Dorzolamide**, a carbonic anhydrase inhibitor effective in the treatment of ocular hypertension and glaucoma, is used to prevent vision loss caused by increased intraocular pressure and contains a thiopyran structure (Gharge & Alegaon, 2024).




**Thiazepine**

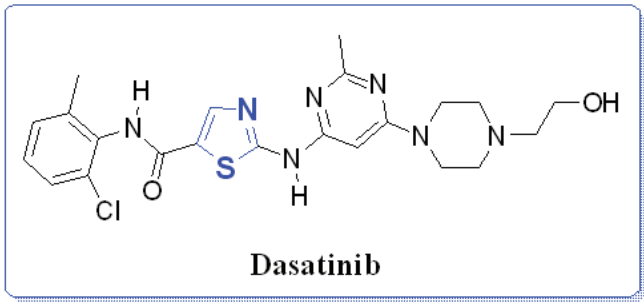
Thiazepine is a seven-membered HCs containing both sulfur and nitrogen atoms within its ring structure. The sulfur atom adjacent to the nitrogen is defined as 1,2-thiazepine, while a 1,4-thiazepine core also exists. In newly designed drug molecules, the fusion of the 1,4-thiazepine isomer with phenyl and biphenyl rings enhances its bioactivity and supports its classification within the heterocyclic compounds class.

**Diltiazem**, a vasodilator and calcium channel blocker used in the treatment of hypertension, myocardial infarction, stroke, and angina pectoris, reduces vascular pressure by optimizing oxygen delivery and blood flow (Gharge & Alegaon, 2024; Kerru et al., 2020).



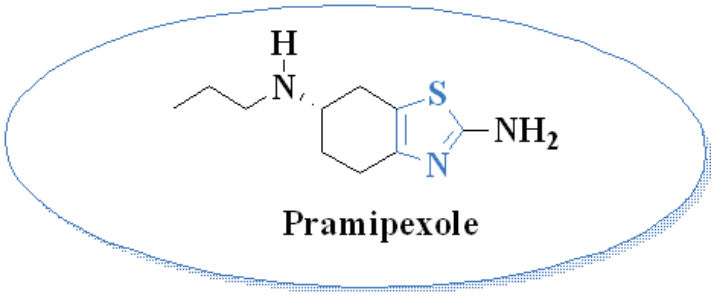

Diltiazem

**Dasatinib**, an active compound containing the **thiazole** group, inhibits tyrosine kinase activity and is used in the treatment of chronic myeloid leukemia. It is believed to reduce resistance to the drug imatinib (Gharge & Alegaon, 2024).

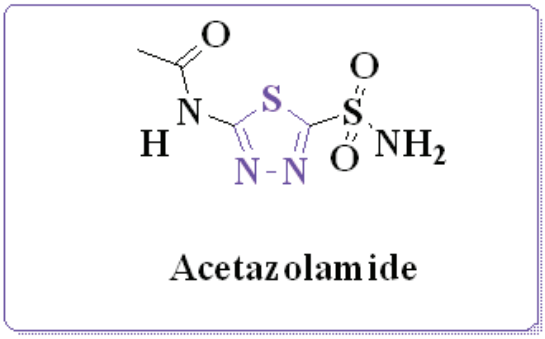



Dasatinib

The deficiency of dopamine, responsible for motor control in the brain, leads to slowed movements and tremors in the legs. **Pramipexole,** designed as a dopamine receptor agonist and containing a **thiazole** ring in its structure, is used in the treatment of Parkinson's disease (Gharge & Alegaon, 2024; Ungureanu et al., 2024).



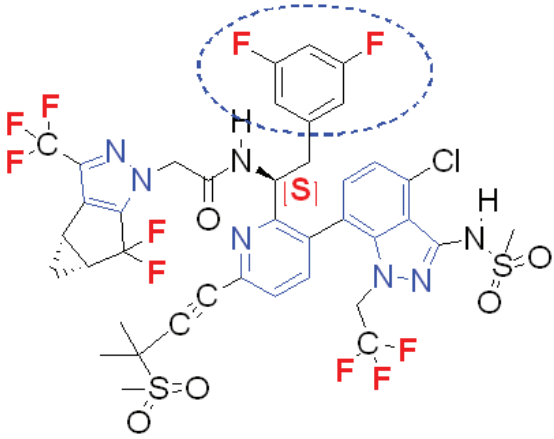

**Acetazolamide**, which contains an active 1,3,4-thiadiazole ring in its pharmaceutical structure, is a carbonic anhydrase inhibitor with anticonvulsant, antiglaucoma, and diuretic properties. It is used in the treatment of glaucoma, congestive heart failure, epilepsy, and partial paralysis (Gharge & Alegaon, 2024; Kerru et al., 2020).




**Biological Activities of Fluorine-Substituted Heterocyclic Compounds**

In the third quarter of the 20th century, the direct attachment of fluorine atoms to heterocyclic rings or the incorporation of fluorinated groups in reaction design marked a significant advancement in drug development. Due to fluorine's low steric hindrance, high electronegativity, and strong C–F bond, the use of fluorinated heterocyclic compounds in pharmaceutical drugs has become widespread. Improvements in lipophilicity, membrane permeability, and specific receptor affinity observed in the mechanisms of action of fluorinated heterocyclic drugs approved by the FDA have increasingly underscored the importance of these compounds (Rizzo et al., 2023).

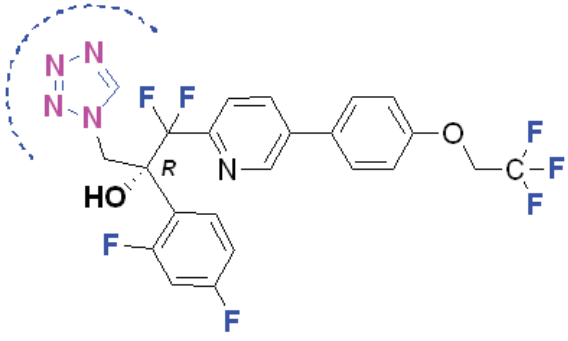
**Lenacapavir**



**Chemical Formula: $C_{39}H_{32}ClF_{10}N_7O_5S_2$**
**Molecular Weight: 968,28**

It is a capsid inhibitor used in the treatment of human immunodeficiency virus type 1 (HIV-1) with a mechanism of action distinct from other antivirals. In viral cycles resistant to reverse transcriptase, protease, and integrase inhibitors, the difluorobenzyl group forms hydrophobic and electrostatic interactions with capsid subunits, stabilizing the capsid structure within the hydrophobic pocket (Rizzo et al., 2023).

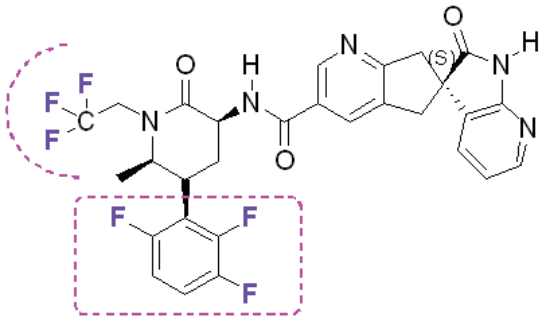
**Oteseconazole**



Chemical Formula: $C_{23}H_{16}F_7N_5O_2$
Molecular Weight: 527,40

It is an antifungal agent that disrupts the cell membrane integrity of pathogenic Candida strains by interacting with cytochrome P450 (CYP51) and reduces the incidence of recurrent vulvovaginal candidiasis (RVVC). The selectivity of this azole metalloenzyme inhibitor for fungal CYP51 is enhanced by the tetrazole group in its structure, minimizing interactions with human cytochrome P450 commonly observed in other antifungals. The phenyl trifluoroethyl ether group and difluoromethyl group within the remaining heterocyclic structure contribute to the metabolite's stability (Rizzo et al., 2023).

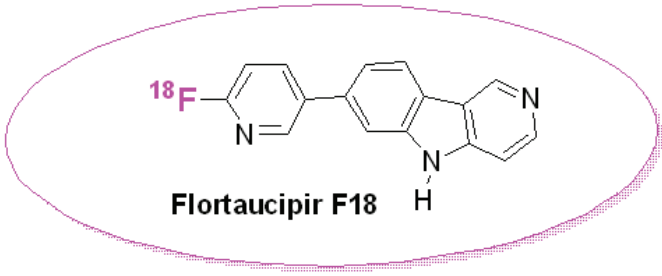
**Atogepant**




Chemical Formula: $C_{29}H_{23}F_6N_5O_3$
Molecular Weight: 603,53

Designed for migraine treatment and attack prevention, the calcitonin gene-related peptide (CGRP) receptor antagonist drug exhibits high affinity due to fluorine substitutions on its phenyl ring, while the presence of fluorine atoms in the structure contributes to low hepatotoxicity. Additionally, unlike other gepant drugs, the steric effect of the trifluoroethyl group on the piperidinone ring is responsible for pharmacokinetic and pharmacodynamic improvements (Rizzo et al., 2023).

**Tauvid**



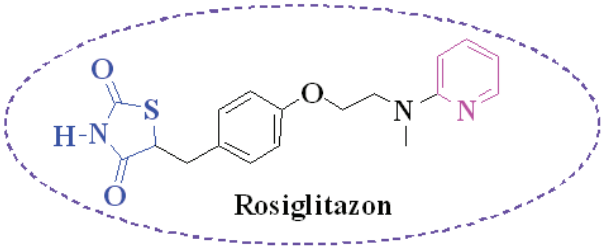

Flortaucipir F18, also known as a positron emission tomography (PET) imaging probe, is the first synthetic molecule developed for Alzheimer's disease (AD). This heterocyclic compound, capable of binding to tau protein, features a fluoropyridine ring responsible for its radioactivity, and its synthesis represents a late-stage fluorination process (Rizzo et al., 2023).

## Pharmacological Effects of Heterocyclic Compounds


### Antidiabetic Activity

Diabetes, characterized by low glucose tolerance and elevated blood glucose levels, is a major health concern in the field of medicine. Nume-

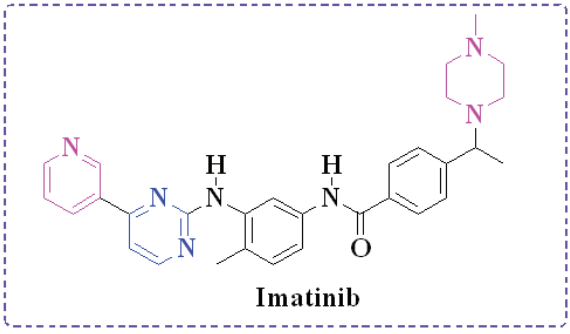
rous antidiabetic agents responsible for glucose homeostasis contain nitrogen- and sulfur-containing heterocyclic compounds in their structures. **Rosiglitazone,** which contains a thiazolidinedione core, is an antihyperglycemic drug that improves insulin sensitivity (Gharge & Alegaon, 2024).



Rosiglitazon

**Anticancer Activity**

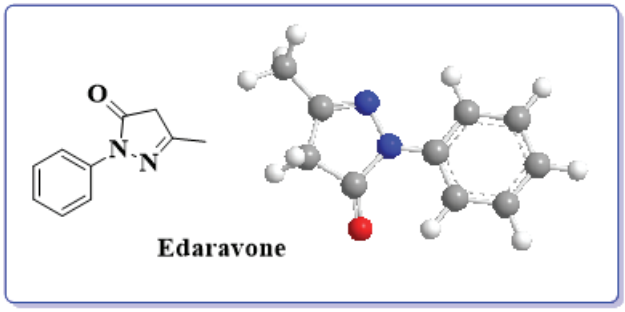
Cancer, one of the pathological conditions with the highest mortality rates, is characterized by abnormal cell growth with the potential to metastasize throughout the body. Among current treatment methods, chemotherapy is defined as the administration of synthetic drugs that inhibit the spread of malignant tumor cells (Mohammed et al., 2023). The toxic effects of these organic compounds on healthy cells, particularly in the treatment of high-risk cancers such as breast, pancreatic, lung, and gastric cancers, have made the development of superior anticancer agents a medical necessity (Pal et al., 2023). In recent years, the incorporation of heterocyclic compounds (HCs) into the design of synthetic agents with improved physicochemical properties for chemotherapy has rendered these structures indispensable molecular scaffolds. Oxadiazole, thiadiazole, imidazole, pyridazine, and pyrazole are among the diverse HCs found in drug structures (Mushtaq et al., 2024).



Imatinib

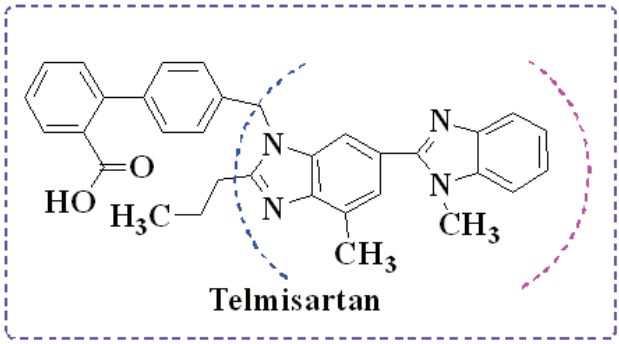
### Antioxidant Activity

Free radicals, considered the primary cause of cellular damage and necrosis, are toxic molecules for metabolism. They lead to diseases by damaging lipids, proteins, and DNA. Numerous natural and synthetic organic compounds known as antioxidants are used to eliminate free radicals from the body. Due to the resonance stability of aromatic ring structures, namely heterocyclic compounds (HCs), the harmful effects of free radicals in the body can be neutralized (Mohammed et al., 2023).




### Antihypertensive Activity

An increase in blood pressure within the arteries is referred to as hypertension. This condition is often thought to be caused by lifestyle, dietary habits, and psychological stress. The drugs used in its treatment operate through various mechanisms of action. There are several compounds with α-blocker activity in which the intermolecular and intramolecular modification properties of heterocyclic compounds (HCs) are utilized (Mohammed et al., 2023).



## Conclusion

Since their discovery, heterocyclic compounds (HCs) have remained one of the dynamically evolving research topics in organic chemistry. Their broad spectrum of applications, including dyes, corrosion inhibitors, copolymers, disinfectants, and agrochemicals, has driven sustained interest in their synthesis. In addition to their diverse structural frameworks and intriguing reaction conditions, the wide range of biological activities exhibited by HCs offers a comprehensive perspective for their evaluation in medicinal chemistry. Nitrogen- and sulfur-containing heterocycles, which are among the essential scaffolds in pharmaceutical chemistry, possess exceptional capabilities for interacting with various biological targets, making them highly attractive candidates for drug development. Structural modifications, particularly the incorporation of active substituents such as fluorine, represent a promising strategy in drug discovery and optimization. Extensive research has demonstrated that heterocycles such as triazole, imidazole, pyrrole, pyrazole, benzimidazole, pyrimidine, pyridazine, thiirane, thietane, thiophene, thiopyran, and thiazepine exhibit a range of therapeutic properties, including anti-inflammatory, antifungal, antiviral, antibacterial, antioxidant, anticancer, antimicrobial, antineoplastic, antidiabetic, neurological, and analgesic activities. Consequently, current studies have explored and elucidated the pharmacological profiles of approved drugs and newly designed therapeutic candidates containing nitrogen-, sulfur-, and fluorine-based heterocyclic cores, alongside their interactions with cellular mechanisms. However, the impressive physicochemical characteristics and mechanisms of action of HCs do not guarantee an absence of limitations in therapeutic contexts. Therefore, to enhance the biological safety and efficacy of these compounds, it is essential to improve their pharmacokinetic properties through rigorous investigation and refined synthetic approaches.

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Chapter 9

# NEXT-GENERATION ANTIHYPERLIPIDEMIC DRUGS

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## NEXT-GENERATION ANTIHYPERLIPIDEMIC DRUGS

In recent years, lipid-lowering interventions have assumed greater significance in strategies aimed at preventing and controlling cardiovascular disease. Although statins have long served as the primary means of lowering LDL-C levels, sufficient efficacy is not always achieved in some patients. Therefore, new generation antihyperlipidemic drugs have been developed. By inhibiting the breakdown of LDL receptors (LDL-R), PCSK9 inhibitors significantly lower LDL-C levels by approximately 50–60%, and their use has become increasingly prevalent among high-risk patients whose LDL-C remains elevated despite statin treatment (O'Donoghue et al., 2022; Sabatine, 2019). Additionally, agents such as bempedoic acid act specifically in the liver to inhibit cholesterol biosynthesis, providing an additional lipid-lowering effect (Biolo et al., 2022; Chandramahanti, Patel, & Farzam, 2025). CETP inhibitors like obicetrapib contribute to improved lipid profiles by enhancing levels of HDL-C (high-density lipoprotein cholesterol) while reducing LDL-C levels (Nicholls et al., 2024). Among triglyceride-lowering agents, volanesorsen has shown efficacy particularly in rare lipid metabofor patlism disorders such as familial chylomicronemia syndrome, effectively controlling elevated triglyceride levels (Esan & Wierzbicki, 2020; Witztum et al., 2019). These next-generation drugs offer promising prospects for more effective cardiovascular risk management and improved patient adherence to treatment (Ballantyne et al., 2023).

### 1. PCSK9 INHIBITORS – ALIROCUMAB AND EVOLOCUMAB

PCSK9 is a liver-derived protease that plays an essential role in regulating the body's cholesterol homeostasis. It demonstrates the capacity to attach to LDL receptors (LDLR) on liver cells. When the PCSK9-LDLR complex is internalized via endocytosis, LDLR is degraded in lysosomes. LDLR serves as the main receptor for removing LDL from the circulation; thus, its downregulation by PCSK9 results in increased concentrations of LDL-C in the bloodstream. PCSK9 inhibitors work by preventing PCSK9 from binding to LDLR, thereby increasing the number of LDLRs available for clearing LDL from the bloodstream and reducing LDL-C levels. By inhibiting LDLR degradation, they facilitate the transfer of high plasma LDL cholesterol to hepatocytes, resulting in decreased LDL-C levels in the blood while increasing its concentration in hepatocytes (Schulz & Schlüter, 2017).

The ODYSSEY COMBO I trial, a Phase III randomized, double-blind, placebo-controlled study, investigated the efficacy and safety of alirocumab in individuals at elevated cardiovascular risk who were unable to

attain target LDL-C levels despite receiving the highest tolerated doses of statins. A total of 316 participants were enrolled and randomly assigned in a 2:1 ratio to receive either alirocumab (n = 209) or placebo (n = 107). After 24 weeks of treatment, alirocumab resulted in a substantial 48.2% reduction in LDL-C, with 75% of patients achieving concentrations below 70 mg/dL. The therapy also led to notable reductions in apolipoprotein B (36.7%) and lipoprotein(a) (20.5%). The overall safety profile was comparable between groups, though mild injection site reactions (5.3%) and nasopharyngitis (7.2%) occurred more frequently in those receiving alirocumab. Importantly, the incidence of major cardiovascular events did not differ significantly between the treatment and placebo groups. These findings support the role of alirocumab as an effective and well-tolerated option for lowering LDL-C in high-risk cardiovascular populations (Kereiakes et al., 2015).

Findings from the ODYSSEY Outcomes trial offer valuable insights into the long-term therapeutic effects of alirocumab. A total of 8,242 individuals, representing 43.5% of the entire study cohort, underwent follow-up monitoring for a duration ranging from three to five years. The median follow-up time of 3.3 years was recorded, with 1,574 individuals being followed for a duration of four to five years. No safety signals were detected throughout the study, and the persistent efficacy of alirocumab was demonstrated. These data underline the prolonged safety profile and patient tolerability of fully human-derived PCSK9 antibodies, highlighting the importance of early initiation of therapy (Goodman et al., 2023).

The FOURIER study represents the first successfully completed cardiovascular outcomes trial investigating the efficacy of evolocumab. This large-scale trial enrolled a total of 27,564 patients diagnosed with clinically evident atherosclerotic cardiovascular disease (ASCVD), including individuals with a documented history of myocardial infarction, non-hemorrhagic stroke, or symptomatic peripheral arterial disease. Eligibility criteria required participants to have an LDL cholesterol (LDL-C) level of ≥70 mg/dL or a non-HDL cholesterol level of ≥100 mg/dL despite receiving optimized, stable lipid-lowering therapy, which ideally consisted of a high-intensity statin regimen equivalent to at least 20 mg of atorvastatin, with or without the addition of ezetimibe. Participants were randomized to receive either 140 mg of evolocumab every two weeks, 420 mg monthly, or a placebo injection that was visually indistinguishable from the active treatment. Plasma LDL-C concentrations were primarily determined using the Friedewald formula; however, for values below 40 mg/dL, preparative ultracentrifugation was employed to ensure accuracy. The main efficacy outcome of the trial was the incidence of major cardiovascular events, assessed as a composite measure comprising cardiovas-

cular mortality, myocardial infarction, stroke, coronary revascularization procedures, or admissions due to unstable angina (Sabatine et al., 2016).

The FOURIER-OLE study enrolled a total of 6,635 participants, with 3,355 individuals originally assigned to the evolocumab arm and 3,280 to the placebo group in the initial trial. The median duration of follow-up within the FOURIER-OLE extension was 5.0 years. When combined with the original FOURIER study period, patients' maximum exposure to evolocumab extended up to 8.4 years. By week 12 of FOURIER-OLE, the median LDL cholesterol level in the evolocumab-treated cohort was 30 mg/dL, with 63.2% of these patients achieving LDL-C concentrations below 40 mg/dL. Extended use of evolocumab was not associated with a higher incidence of severe adverse effects, musculoskeletal complaints, new-onset diabetes, hemorrhagic stroke, or neurocognitive issues relative to the placebo group in the original trial, and these safety outcomes remained stable throughout the follow-up period. During the extension phase, patients initially randomized to evolocumab experienced a 15% reduction in the risk of major cardiovascular events, a 20% lower combined risk of cardiovascular death, myocardial infarction, or stroke (P=0.003), and a 23% decrease in cardiovascular mortality relative to placebo recipients. The FOURIER open-label extension currently represents the most prolonged follow-up available for PCSK9 inhibitor therapy, underscoring the benefits of early treatment initiation and affirming the long-term safety of evolocumab over more than eight years of continuous use (O'Donoghue et al., 2022).

**Table 1**. *Comparison of Major Clinical Trials on PCSK9 Inhibitors*

| Study Name | Drug | Study Design | Population | Duration | Main Outcomes | Safety Profile |
|---|---|---|---|---|---|---|
| **ODYSSEY COMBO I** | Alirocumab | A double-blind, placebo-controlled, randomized Phase III study | 316 high-risk patients with uncontrolled LDL-C despite max statin therapy | 24 weeks | ↓ LDL-C by 48.2%, 75% reached <70 mg/dL; ↓ ApoB by 36.7%, ↓ Lp(a) by 20.5% | Similar to placebo; ↑ injection site reactions (5.3%), ↑ nasopharyngitis (7.2%) |
| **ODYSSEY OUTCOMES** | Alirocumab | A randomized, double-blind study with extended follow-up | 8,242 patients (43.5% of study pop.); median follow-up: 3.3 years | Up to 5 years (median 3.3 yrs) | Sustained LDL-C reduction; long-term safety and efficacy confirmed | No new safety signals detected |
| **FOURIER** | Evolocumab | A Phase III CVoutcomes trial designed as a randomized, double-blind, placebo-controlled study | 27,564 ASCVD patients (MI, stroke, PAD) | Median 2.2 years | ↓ LDL-C to ~30 mg/dL; ↓ CV events: MI, stroke, revascularization | Well tolerated; no ↑ in serious AEs, diabetes, neurocognitive effects |
| **FOURIER-OLE** | Evolocumab | Open-label extension of FOURIER | 6,635 patients (from original trial) | Median 5.0 years; up to 8.4 yrs | ↓ LDL-C to 30 mg/dL; 63.2% reached <40 mg/dL; ↓ 15% CV events, ↓ 20% CV death/MI/stroke, ↓ 23% CV death | No safety signal increase over time |

PCSK9 inhibitors and statins share some similarities but also present key differences. Both treatments reduce plasma LDL-C levels by around 60% at their highest doses. However, statins are more effective in lowering plasma triglyceride levels compared to PCSK9 inhibitors (25–35% versus approximately 15%). Conversely, the reduction in plasma apolipoprotein B levels, which reflects all atherogenic particles, is similar with both therapies (about 50%). While PCSK9 inhibitors reduce plasma lipoprotein(a) levels by approximately 25%, statins have no significant effect on this parameter. In addition, statins have been shown to possess anti-inflammatory properties, evidenced by reduced plasma C-reactive protein (CRP) levels, whereas PCSK9 inhibitors do not affect high-sensitivity CRP concentrations. How these differential effects beyond LDL-C lowering translate into clinical risk reduction remains an open question (Sabatine, 2019).

**Table 2.** *Additional Comparison Points Between PCSK9 Inhibitors and Statins*

| Parameter | PCSK9 Inhibitors | Statins |
|---|---|---|
| LDL-C Reduction (max dose) | ~ 60% | ~ 60% |
| Triglyceride Reduction | ~15% | 25–35% |
| ApoB Reduction | ~50% | ~50% |
| Lipoprotein(a) Reduction | ~25% | No significant effect |
| Effect on CRP (inflammation) | No effect | ↓ CRP levels |

## 2. APOC3 INHIBITOR-VOLANESORSEN

Physiologically, apolipoprotein C3 (apoC3) regulates triglyceride (TG) levels by inhibiting the breakdown of very-low-density lipoproteins (VLDL) and chylomicrons, either through lipoprotein lipase (LPL) activity or independently of LPL. Volanesorsen is an antisense apoC3 inhibitor. Antisense apoC3 inhibitors work by suppressing the production of apoC3 in the liver, thereby reducing elevated TG levels. Increased apoC3 levels have been associated with hypertriglyceridemia, insulin resistance, metabolic syndrome, and an augmented risk of cardiovascular disease. Volanesorsen has been effective in reducing triglyceride levels by approximately 70–80%. Clinical trials have demonstrated that volanesorsen effectively reduces TG levels in patients with Familial Chylomicronemia Syndrome (FCS) and hypertriglyceridemia (Esan & Wierzbicki, 2020).

### The Role of APOC3 in Plasma Triglyceride Metabolism

When plasma TG levels are within the normal range, the body has substantial capacity to handle additional triglyceride input. This input originates from two primary sources: the liver, which produces VLDL, and the intestines, which absorb dietary fat in the form of chylomicrons. Triglyceride removal mainly occurs via a lipoprotein lipase (LPL)-dependent pathway, although a smaller portion is cleared through an LPL-independent route that remains poorly understood. In situations where LPL is deficient or absent, the clearance of TG-rich lipoproteins is severely impaired, resulting in chylomicronemia—defined by plasma triglyceride levels exceeding 880 mg/dL (10 mmol/L). While apoC3 was traditionally believed to regulate triglyceride metabolism by inhibiting LPL activity, recent findings suggest that it also exerts a strong inhibitory effect on the LPL-independent pathway. Though the precise mechanisms are still being investigated, apoC3 likely interferes with the hepatic intake of TG-

rich lipoprotein remnants, thereby contributing to impaired clearance in this alternative pathway (Gaudet et al., 2014).

**Clinical Studies and Safety Profile of Volanesorsen**

Clinical studies have shown that the most common adverse effects in patients treated with volanesorsen include injection region reactions, reduced platelet counts, nausea, and thrombocytopenia (Paik & Duggan, 2019).

The Phase 3 APPROACH trial assessed the efficacy and safety of volanesorsen in patients with familial chylomicronemia syndrome (FCS) through a randomized, double-blind, placebo-controlled design involving 66 participants. Subjects were assigned to receive a weekly subcutaneous injection of either 300 mg volanesorsen or placebo. Over the 12-week treatment duration, those receiving volanesorsen exhibited marked reductions in triglycerides (77%), VLDL-cholesterol (58%), and apolipoprotein C-III levels (84%), all statistically significant compared to placebo ($p < 0.001$). Despite these improvements, an increase of 136% in LDL-C was recorded, likely as a consequence of intensified lipolytic activity. Thrombocytopenia emerged in approximately half of the volanesorsen-treated patients, with 45.4% experiencing platelet counts under 100,000/μL and two individuals falling below 25,000/μL. No such events were reported in the placebo group. Injection site reactions were observed in 60.6% of patients receiving volanesorsen, whereas none were reported among placebo recipients (Witztum et al., 2019).

The COMPASS trial was a multicenter, randomized, double-blind, placebo-controlled Phase 3 study that included 133 patients diagnosed with multifactorial chylomicronemia, all having baseline triglyceride levels of at least 500 mg/dL. Participants were assigned to receive volanesorsen or placebo under two distinct dosing schedules. After three months of treatment, those in the volanesorsen arm demonstrated a mean reduction in triglyceride levels of 71.2%, in contrast to a minimal 0.9% decrease observed in the placebo group. Apolipoprotein C3 (ApoC3) concentrations dropped by 76.1%, accompanied by a notable 95.5% rise in LDL cholesterol attributed to increased lipolysis ($p < 0.001$ vs. placebo). Thrombocytopenia was observed in 13% of patients treated with volanesorsen, including one individual whose platelet count declined below 50,000/μL, whereas the incidence in the placebo cohort was 5%. Importantly, all five cases of acute pancreatitis were reported exclusively in the placebo group (Gouni-Berthold et al., 2021).

In another study, four patients with FCS and four matched healthy controls were included to assess changes in platelet counts. By week 12 of the treatment, platelet levels showed a 30% variation, which further increased

to 34% by week 36. Thrombin production stayed within normal limits, with no notable differences found between the patient group and the control group. Platelet aggregation tests also showed the same values within the expected range, with no significant differences compared to healthy controls. This research presented the initial indication that administration of volanesorsen did not cause significant changes in overall hemostatic balance or platelet activity, as assessed by thrombin generation assays, in individuals diagnosed with familial chylomicronemia syndrome (FCS). These findings suggest that volanesorsen treatment is unlikely to adversely affect coagulation pathways or platelet function, thereby supporting its safety profile in managing this rare lipid disorder. Further longitudinal studies are warranted to confirm these observations over extended treatment periods (Calcaterra et al., 2024).

**Table 3**. *Comparative table of major clinical studies with volanesorsen*

| Study | Population | Design | Intervention | Key Outcomes | Adverse Effects |
|---|---|---|---|---|---|
| **APPROACH (2019)** | 66 patients with FCS | Phase 3, RCT, double-blind, placebo-controlled | Volanesorsen 300 mg weekly | ↓ TG by 77%, ↓ VLDL-C by 58%, ↓ ApoC3 by 84%, ↑ LDL-C by 136% | Thrombocytopenia in 45.4% (2 with platelets <25,000/μL), Injection site reactions in 60.6% |
| **COMPASS (2021)** | 133 patients with multifactorial chylomicronemia (TG ≥500 mg/dL) | Phase 3, RCT, double-blind, placebo-controlled | Volanesorsen | ↓ TG by 71.2%, ↓ ApoC3 by 76.1%, ↑ LDL-C by 95.5% | Thrombocytopenia in 13%, 1 with platelets <50,000/μL, 5 acute pancreatitis cases (all in placebo group) |
| **Calcaterra (2024)** | 4 FCS patients, 4 matched controls | Observational, prospective | Volanesorsen | Platelet count ↓ 30–34% over 36 weeks; no significant changes in thrombin generation or aggregation | No significant changes in overall hemostasis or platelet function compared to controls |

## Application

Volanesorsen is given through subcutaneous injections, with an initial recommended dose of 285 mg once per week for a duration of three months. Following this period, the dosing interval is adjusted to 285 mg every other week. Therapy discontinuation is recommended for patients who do not demonstrate a minimum 25% reduction in serum triglycerides or whose triglyceride levels do not fall below 22.6 mmol/L after receiving

285 mg of volanesorsen weekly for three months (Esan & Wierzbicki, 2020).

## 3. BEMPEDOIC ACID

Bempedoic acid is an important medication for lowering lipids, commonly prescribed to patients with high levels of low-density lipoprotein cholesterol (LDL-C). The U.S. Food and Drug Administration has authorized the use of bempedoic acid as an add-on to the highest tolerated statin therapy for patients with atherosclerotic cardiovascular disease (ASCVD) and heterozygous familial hypercholesterolemia (HeFH) to help lower LDL-C levels. Furthermore, it is regarded as an appropriate option for patients who cannot tolerate statins (Chandramahanti et al., 2025).

Bempedoic acid primarily acts by inhibiting cholesterol synthesis in the liver and is typically not linked to the myotoxic muscle side effects commonly observed with certain other lipid-lowering agents. Its effects on atherosclerotic cardiovascular disease, type 2 diabetes mellitus, and chronic liver conditions have been investigated through randomized clinical trials; however, additional studies are necessary to confirm these findings. Phase III safety assessments have demonstrated that bempedoic acid is generally well tolerated when administered alongside statins, ezetimibe, or PCSK9 inhibitors to help patients reach LDL cholesterol targets (Biolo et al., 2022).

Bempedoic acid is primarily a small molecule aimed at reducing LDL-C levels. Preclinical research has identified a dual mode of action, which includes inhibiting ATP citrate lyase (ACL) and activating adenosine monophosphate-activated protein kinase. However, in humans, the inhibition of ACL is predominant. The liver-specific inhibition of ATP citrate lyase by bempedoic acid reduces LDL-C levels and mitigates atherosclerosis. Bempedoic acid functions as a prodrug that becomes active through the action of long-chain acyl-CoA synthetase 1 (ACSVL1). Inhibition of ACL increases the number of LDL-R, thereby reducing LDL-C levels. Importantly, since skeletal muscle cells lack the ACSVL1 enzyme, bempedoic acid remains in its inactive form, preventing the myotoxic side effects typically observed with statin use (Pinkosky et al., 2016).

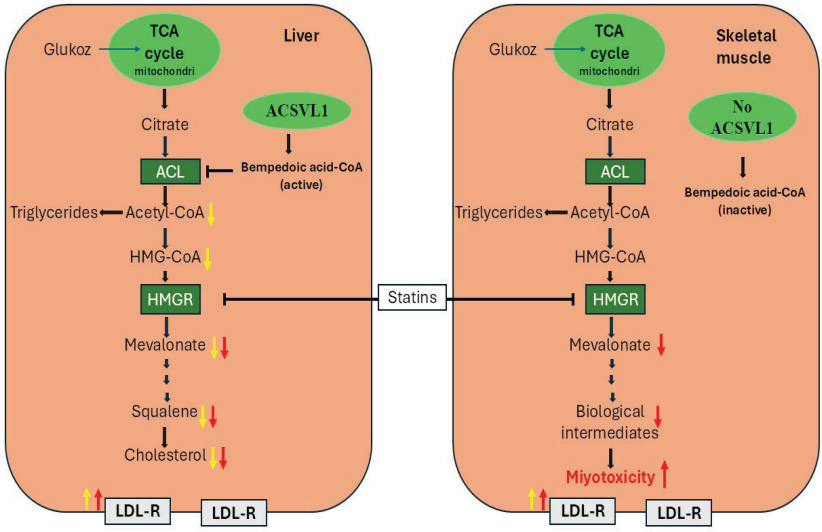


**Figure 1. Bempedoic Acid Mode of Action within the Liver**. *In the liver, bempedoic acid is transformed into its active metabolite, bempedoic acid-CoA, through the enzyme ACSVL1. This active metabolite inhibits ACL, a crucial enzyme in the cholesterol biosynthesis pathway. Inhibition of ACL reduces cholesterol production in hepatocytes and upregulates LDL-R expression. An elevated amount of LDL receptors (LDL-R) boosts the removal of LDL particles from the bloodstream, resulting in lowered plasma LDL-C concentrations. In skeletal muscle cells, the ACSVL1 enzyme is absent; therefore, bempedoic acid cannot be converted into its active form. Consequently, it does not affect cholesterol synthesis in muscle tissue, thereby avoiding the myotoxicity risk typically linked to statin therapy. The* **yellow arrows** *represent the effects of bempedoic acid on cholesterol synthesis, while the* **red arrows** *indicate action of statins (Pinkosky et al., 2016).*

## 4. DIACYLGLYCEROL ACYLTRANSFERASE (DGAT) INHIBITION

Intestinal DGAT1 is an enzyme essential for fat absorption and triglyceride synthesis. At the outset, its function in lipid metabolism positioned it as a potential treatment target for FCS, yet advancement was constrained due to the absence of confirming evidence from rare family-based studies and Mendelian randomization that demonstrated effects on atherosclerotic cardiovascular disease. Pradigastat (Novartis, Basel, Switzerland) and AZD7687 (AstraZeneca, London, UK) are both oral small-molecule inhibitors of DGAT1; however, AZD7687 was associated with intolerable gastrointestinal side effects (Hegele & Tsimikas, 2019).

In a recent study involving six patients diagnosed with familial chylomicronemia syndrome (FCS) treated with pradigastat, fasting triglyceride concentrations were lowered by as much as 70%, with no significant safety issues reported (Meyers, Tremblay, et al., 2015). Additionally, an early-phase trial including 106 overweight or obese subjects administered varying increasing doses of pradigastat demonstrated reductions in postprandial triglycerides, glucose levels, and insulin secretion, alongside an elevation in postprandial glucagon-like peptide-1 concentrations. Gastrointestinal side effects such as diarrhea and nausea were evident at a 10 mg dose; however, reducing dietary fat intake improved tolerability (Meyers, Amer, Majumdar, & Chen, 2015), although these adverse effects still pose limitations for its development and widespread use.

## 5. MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN (MTTP) INHIBITOR – LOMITAPIDE

Microsomal triglyceride transfer protein (MTTP) is a protein that facilitates the transfer of triglycerides onto apolipoprotein B (ApoB) in the intestine and liver, thereby promoting the synthesis of VLDL and chylomicrons. The inhibition of MTTP suppresses the synthesis of chylomicrons and VLDL, leading to a decrease in LDL-C levels. While conventional treatments aim to lower plasma LDL-C concentrations by enhancing its clearance, in patients with homozygous familial hypercholesterolemia (HoFH), their effectiveness is often limited Lomitapide, a newly authorized drug, is prescribed for managing hyperlipidemia in adult individuals diagnosed with HoFH. It is an oral inhibitor of MTTP, expressed chiefly inside the endoplasmic reticulum lumen of both hepatocytes and enterocytes. Lomitapide exerts its lipid-lowering effect through a unique mechanism that bypasses the classical LDL receptor-mediated clearance pathway targeted by statins and other conventional therapies, enabling effective LDL-C reduction (Munkhsaikhan et al., 2024).

Studies have demonstrated that lomitapide can decrease LDL-C concentrations by nearly 50%. In a key Phase 3 trial supporting its approval for HoFH patients, lomitapide was given to 29 participants. Over a 26-week treatment period, adding lomitapide to standard therapy led to about a 50% decrease in LDL-C and ApoB levels, as well as a 15% reduction in lipoprotein(a) concentrations (Cuchel et al., 2013).

The most significant adverse effect associated with lomitapide is hepatic steatosis. It may also lead to elevated transaminase levels. Therefore, it is recommended to assess baseline levels of ALT, AST, ALP, and total bilirubin before initiating treatment and to monitor ALT and AST levels during therapy. Lomitapide should be co-administered with other

hepatotoxic medications only with caution, given the increased risk of hepatic adverse effects. In the phase 3 study, ALT and/or AST levels exceeded three times the upper limit of normal (ULN) in at least one instance in ten participants. Among them, four exhibited ALT levels exceeding five times the ULN, with one individual also showing a similar rise in AST levels. These increases were detected following the administration of lomitapide at doses of 10 mg, 20 mg, 40 mg, and 60 mg.. No participants permanently stopped treatment because of elevated liver function test (LFT); all increases were managed via dose reduction or temporary discontinuation, in accordance with the study protocol. Notably, three of the four participants with LFT levels >5× ULN reported alcohol intake exceeding the protocol allowance. None of the participants showed any rises in bilirubin or alkaline phosphatase levels (Cuchel et al., 2013).

Moreover, if a very low-fat diet is not followed, lomitapide may cause intestinal fat accumulation, resulting in abdominal pain, diarrhea, and steatorrhea. Additionally, failure to supplement vitamins can lead to deficiencies in fat-soluble vitamins (Khoury, Brisson, Roy, Tremblay, & Gaudet, 2019).

Lomitapide therapy should be initiated orally at a dose of 5 mg daily. Depending on efficacy and tolerability, the dose may be doubled every two weeks, up to a maximum of 60 mg/day (Cuchel et al., 2013). Lomitapide should be administered in a fasting state, ideally at least two hours post-evening meal. Because it impairs the absorption of fat-soluble vitamins, daily supplementation with one capsule of 400 IU vitamin E and one capsule of omega fatty acids is required throughout the course of therapy. These supplements are preferably taken in the morning (Kameyama et al., 2019).

## 6. ANTISENSE APOLIPOPROTEIN B100 INHIBITOR – MIPOMERSEN

Mipomersen, a second-generation antisense oligonucleotide directed against apolipoprotein B100, received approval in January 2013 for the management of HoFH. It exerts its effect by inhibiting the translation of apolipoprotein B mRNA, thereby reducing the hepatic synthesis of apolipoprotein B100. This reduction leads to decreased hepatic VLDL production and ultimately lower plasma LDL levels. Mipomersen is administered via subcutaneous injection at a weekly dose of 200 mg (Rader & Kastelein, 2014).

Fifty-one patients with HoFH were enrolled in a clinical trial; among them, 34 patients received mipomersen while 17 were given placebo, both as adjuncts to standard therapy, and were followed for 26 weeks.

Mipomersen treatment led to a 21% reduction in LDL-C levels and a 24% reduction in apolipoprotein B levels compared to placebo. Additionally, reductions were observed in non-HDL-C (21.6%), triglycerides (17%), and lipoprotein(a) [Lp(a)] levels (23%). In contrast, increases of 11.2% in HDL-C and 3.9% in apolipoprotein A-I levels were reported (Raal et al., 2010). Mipomersen has also been studied in individuals with heterozygous familial hypercholesterolemia (HeFH). In a randomized, double-blind, placebo-controlled trial, participants receiving the highest tolerated dose of statin therapy were administered weekly subcutaneous doses of either 200 mg mipomersen or a matching placebo.After 26 weeks, patients in the mipomersen group experienced a 33% reduction in LDL-C levels compared to placebo. Notable reductions were also observed in apolipoprotein B (26%), triglycerides (14%), and lipoprotein(a) (21%), whereas HDL-C levels remained largely unchanged (Stein et al., 2012). A follow-up extension study demonstrated that these beneficial effects of mipomersen could be maintained for at least two years (Santos et al., 2015).

In Phase 3 clinical trials, the most reported adverse events were injection region reactions, such as pain, erythema, and pruritus. Approximately 30% of patients reported influenza-like symptoms—such as chills, myalgia, arthralgia, fatigue, and general malaise—within two days following injection (Stein et al., 2012). Data from phase 3 trials also revealed that patients treated with mipomersen exhibited significantly greater elevations in alanine aminotransferase (ALT) compared to controls (McGowan et al., 2012). Mipomersen should be used with caution when co-administered with known hepatotoxic substances such as methotrexate, tamoxifen, isotretinoin, or alcohol due to the potential risk of liver toxicity. (Stein et al., 2012). To minimize adverse effects, a randomized controlled trial evaluated the safety and efficacy of a reduced dosing regimen of mipomersen, administered three times weekly at 70 mg per injection. The results indicated that this dosing regimen was associated with fewer side effects and improved treatment tolerability (Reeskamp et al., 2019).

## 7. CETP INHIBITOR – OBICETRAPIB

Obicetrapib, a targeted inhibitor of cholesteryl ester transfer protein (CETP), is undergoing clinical evaluation for its capacity to reduce LDL-C concentrations and potentially lower the risk of major adverse cardiovascular events. The **TULIP study**, conducted in Denmark and the Netherlands with 364 participants exhibiting mild dyslipidemia, evaluated the CETP-inhibitory effects of obicetrapib. 12-week treatment with 10 mg/day obicetrapib in combination with moderate-intensity statins resulted in up to a 50% greater lowering of LDL-C compared to statin mono-

therapy (Hovingh et al., 2015).

In a separate randomized, double-blind, placebo-controlled study (NCT04753606) including 120 patients with dyslipidemia (median LDL-C of 88 mg/dL) undergoing high-intensity statin therapy, the lipid-lowering effects of obicetrapib were evaluated. After 8 weeks of treatment with either 5 mg or 10 mg doses, obicetrapib demonstrated a significant decrease in median LDL cholesterol levels by up to 51% ($P < 0.0001$) compared to placebo. Furthermore, the treatment produced notable reductions in apolipoprotein B levels (up to 30%) and non-HDL cholesterol (up to 44%), alongside a marked elevation in HDL cholesterol concentrations (up to 165%). The medication was well tolerated overall, suggesting its promising role for patients at elevated cardiovascular risk (Nicholls et al., 2022).

In a randomized, double-blind Phase 2 study, patients with LDL cholesterol levels above 70 mg/dL and triglycerides below 400 mg/dL, all stable on high-intensity statin therapy, were treated for 12 weeks with either a combination of 10 mg obicetrapib and 10 mg ezetimibe ($n = 40$), 10 mg obicetrapib alone ($n = 39$), or placebo ($n = 40$). The primary analysis included 97 participants, with a mean age of 62.6 years and 63.9% male. LDL-C reductions from baseline to week 12 were 63.4% in the combination therapy group, 43.5% in the obicetrapib monotherapy group, and 6.35% in the placebo group ($P < 0.0001$ compared to placebo). Within the combination arm, all patients reached LDL-C levels below 100 mg/dL, 93.5% achieved levels under 70 mg/dL, and 87.1% attained levels below 55 mg/dL. Both active treatment regimens significantly lowered non-HDL cholesterol, apolipoprotein B, total LDL particle count, and small LDL particle concentrations. Obicetrapib was generally well tolerated, with no serious adverse safety events reported throughout the study (Ballantyne et al., 2023).

**Table 4**. *Overview and Comparative Summary of Key Clinical Trials on Obicetrapib*

| Study Name | Patient Population | Treatment Duration | Obicetrapib Dose | Key Findings | Safety Profile |
|---|---|---|---|---|---|
| **TULIP (2015)** | 364 patients with mild dyslipidemia | 12 weeks | 10 mg + moderate-intensity statin | Up to 50% additional LDL-C reduction compared to statin monotherapy | Well tolerated |
| **NCT04753606 (2022)** | 120 dyslipidemic patients on high-intensity statins | 8 weeks | 5 mg / 10 mg | Up to 51% LDL-C, 30% ApoB, and 44% non-HDL-C reduction; up to 165% HDL-C increase | Acceptable safety profile |
| **Ballantyne et al. (2023)** | A total of 97 patients having LDL-C above 70 mg/dL under consistent statin treatment | 12 weeks | 10 mg ± 10 mg ezetimibe | LDL-C reduction of 63.4% (combination) and 43.5% (monotherapy); 87.1% of patients achieved LDL-C <55 mg/dL | Well tolerated |
| **BROOKLYN (2024)** | 354 adults with HeFH and uncontrolled LDL-C despite therapy | 52 weeks | 10 mg | 36.3% LDL-C reduction at week 12, 41.5% at week 52; over 50% of patients achieved LDL-C below 70 mg/dL | Similar to placebo |
| **BROADWAY (2024)** | 2,532 patients with ASCVD and/or HeFH and uncontrolled LDL-C | 12 weeks | 10 mg | 33% LDL-C reduction; significant improvements in ApoB, Lp(a), HDL-C, non-HDL-C, total cholesterol, and triglycerides | Similar to placebo |
| **Harada-Shiba et al. (2024)** | 102 Japanese patients with dyslipidemia on statins | 8 weeks | 2.5 / 5 / 10 mg | At 10 mg dose: LDL-C ↓45.8%, ApoB ↓29.7%, non-HDL-C ↓37%, HDL-C ↑159% | Well tolerated; no major safety issues |

The BROOKLYN study included 354 adults with heterozygous familial hypercholesterolemia (HeFH) whose LDL-C levels remained uncontrolled despite standard lipid-lowering therapies. Participants were randomized in a 2:1 ratio to receive 10 mg obicetrapib or placebo. The primary endpoint, change in LDL-C at week 12, demonstrated a 36.3% reduction with obicetrapib treatment ($P < 0.0001$). This effect was sustained through week 52, with a 41.5% reduction. More than 50% of participants achieved LDL-C levels below 70 mg/dL. Obicetrapib also significantly lowered non-HDL-C, ApoB, and Lp(a), and exhibited a favorable safety profile similar to that of placebo. The BROADWAY study included 2,532

adults with ASCVD and/or HeFH whose LDL-C levels were inadequately controlled despite current lipid-lowering therapies. In a 2:1 randomization scheme, participants received either 10 mg of obicetrapib or placebo. At week 12, LDL-C levels were reduced by 33% in the obicetrapib group ($P < 0.0001$). Secondary endpoints included changes in ApoB, Lp(a), ApoA1, HDL-C, non-HDL-C, total cholesterol, and triglycerides—all of which showed favorable responses. The drug demonstrated well tolerability, with a safety profile similar to that of the placebo (Nicholls et al., 2024). These findings support obicetrapib as a promising treatment option for patients who fail to reach target LDL-C levels with existing therapies.

A randomized, placebo-controlled Phase 2 clinical trial with double-blinding conducted in Japan evaluated 102 patients with dyslipidemia who were stable on statin therapy. Eligibility criteria included LDL-C levels above 70 mg/dL or non-HDL-C greater than 100 mg/dL, along with triglyceride levels less than 400 mg/dL. Patients were randomized to receive daily doses of 2.5 mg, 5 mg, or 10 mg obicetrapib or placebo for 8 weeks. Obicetrapib demonstrated dose-dependent reductions in LDL-C, apoB, and non-HDL-C levels, with the 10 mg dose yielding reductions of 45.8%, 29.7%, and 37%, respectively. HDL-C levels increased by 159%. All changes were statistically significant compared to placebo ($P < 0.0001$). Pharmacokinetic analyses indicated that obicetrapib is nearly eliminated from the body within approximately 4 weeks. The therapy was generally well tolerated, and no major adverse safety signals were observed (Harada-Shiba et al., 2024).

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Ramazan BİLGİN

Chapter 10

# A MULTIDIMENSIONAL BIOCHEMICAL INVESTIGATION OF ALZHEIMER'S DISEASE: ETIOLOGICAL FACTORS AND THERAPEUTIC AGENTS

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## Introduction

Alzheimer's disease (AD) is a common neurodegenerative disorder affecting around 40 million people worldwide, with cases expected to exceed 100 million by 2050 (Gupta et al., 2020). The preclinical stage of AD is asymptomatic, although biomarker studies have revealed early pathological changes, such as amyloid plaques and neurofibrillary tangles in the hippocampus and cortex. This stage may last for years without impairing daily functioning. In mild AD, memory loss, language difficulties, mood disturbances, and disorientation emerge (Wattmo et al., 2016). Moderate AD involves worsening cognitive deficits, impaired recognition, and loss of impulse control. In advanced stages, patients exhibit severe motor and cognitive decline, including aphasia, apraxia, and incontinence, and are often bedridden. Hallucinations, circadian rhythm disruption, and behavioural symptoms occur in a substantial number of patients (Krishnamurthy et al., 2024; Wattmo et al., 2016). Late-onset AD is the most common age-related neurodegenerative disorder, marked by progressive memory loss and cognitive decline. Unlike early-onset forms linked to amyloid-β gene mutations, the causes of late-onset AD are complex and not fully understood. It is considered a polygenic disease involving multiple disrupted molecular pathways. Older age is the main risk factor, followed by the APOE ε4 allele and possibly cardiovascular and lifestyle factors. The disease is characterized by abnormal protein buildup (amyloid-β plaques and tau tangles), leading to synapse and neuron loss. These changes disrupt brain networks, including the cholinergic system. Despite many studies, most clinical trials have failed, and no new drugs have been FDA-approved since 2003 (Hampel et al., 2019). Due to its complex nature, personalized and stage-specific treatment approaches may be necessary. However, several hypotheses have been proposed with the experimental data obtained. These hypotheses include the cholinergic hypothesis associated with a decrease in acetylcholine, the amyloid beta (amyloid-β) and tau-protein aggregation hypotheses, which cause neurons to die and disrupt the communication network among themselves, hypotheses related to copper, iron and zinc accumulation, hypotheses related to social isolation and loneliness, hypotheses related to oxidative stress, hypotheses related to mutant ε4 allele of apolipoprotein E (APOE-ε4) (Hampel et al., 2019; Krishnamurthy et al., 2024)(Figure 1). The cholinergic system continues to be regarded as a viable therapeutic avenue in the management of AD treatment. However, the cholinergic hypothesis explains only one aspect of AD. It is now known that AD is a complex disease and many factors such as amyloid plaques, tau proteins, genetic factors and inflammation play a role in the development of AD. Therefore, all these factors that have an impact on the development of AD will be examined in this chapter.

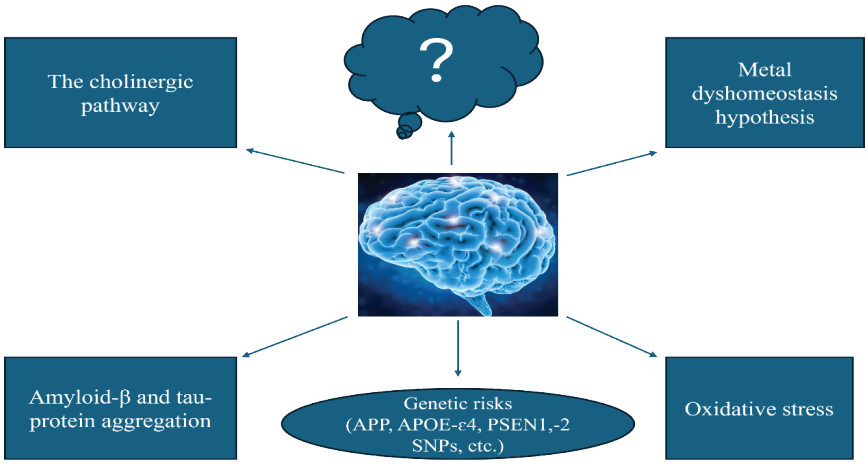


*Figure 1. Major Hypotheses Explaining Alzheimer's Disease*

## Etiological Factors of Alzheimer's Disease

### 1- The cholinergic hypothesis

The cholinergic hypothesis is a theory used to explain neurodegenerative diseases, especially AD. According to this hypothesis: cholinergic synapses are ubiquitous in the human central nervous system. Decreased levels of a neurotransmitter called acetylcholine (ACh) (a chemical that enables communication between nerve cells) in the brain leads to impaired cognitive functions and dementia. Acetylcholine is a chemical that plays a critical role in cognitive functions such as memory, learning, attention and consciousness. As a key neurotransmitter, acetylcholine exerts widespread influence across multiple brain regions, including the cerebral cortex, basal ganglia, and basal forebrain (Hampel et al., 2019; Mesulam, 2013). ACh is synthesized in the cytoplasm of the presynaptic neuron by the enzyme choline acetyltransferase (ChAT), which catalyses the reaction between acetyl coenzyme A (acetyl-CoA) and choline. The synthesized ACh is then stored in synaptic vesicles via vesicular acetylcholine transporters (VAChT) (Purves et al., 2019). The arrival of an action potential at the presynaptic membrane triggers the activation of voltage-dependent $Ca^{2+}$ channels, facilitating an influx of calcium ions. This calcium influx initiates the fusion of vesicles containing acetylcholine (ACh) with the presynaptic membrane, leading to the release of ACh into the synaptic cleft through exocytosis (Kandel et al., 2000). The released ACh then crosses the synaptic cleft and binds to cholinergic receptors located on the postsynaptic membrane. There are two main types of cholinergic receptors: a) Nicotinic receptors, which are ligand-gated

ion channels, primarily mediate fast synaptic transmission by allowing $Na^+$ influx and membrane depolarization. b) Muscarinic receptors, which are G protein-coupled receptors, mediate slower, modulatory responses through intracellular second messenger pathways (Bear et al., 2016). Therefore, it leads to transmitting a signal from one neuron to the other Acetylcholinesterase (AChE)-mediated cleavage of residual acetylcholine produces choline and acetate components that are recuperated and utilized in the reformation of acetyl coenzyme A. Cholinergic neurons which nerve cells that secrete acetylcholine, especially in the basal forebrain, deteriorate or die with age or in diseases such as AD. This impairment weakens the communication between various regions of the brain and leads to a decline in memory, attention and other cognitive skills. The reason for this is prior post-mortem studies have demonstrated that the degeneration of cortical cholinergic innervation is linked to, and likely driven by, the presence of neurofibrillary tangles within the nucleus basalis of Meynert (NBM) (Braak & Del Tredici, 2012; Mesulam, 2013). Cholinergic neurons of the basal forebrain are among the most fragile neuronal populations to neurofibrillary degeneration and tangle formation (Mesulam, 2013). A well-established association also exists between cholinergic dysfunction and amyloid-β pathology. In a seminal study, They identified an inverse correlation between choline acetyltransferase activity, an enzyme critical for acetylcholine synthesis, and the density of neurotic plaques in the post-mortem brains of individuals with AD (PERRY et al., 1978). Moreover, experimental studies in animal models have indicated that cholinergic depletion facilitates amyloid-β accumulation and hyperphosphorylated tau pathology which form protein fibrils that aggregation, thereby exacerbating cognitive deficits (Ramos-Rodriguez et al., 2013). Furthermore, the degeneration of cholinergic neurons may compromise acetylcholine-dependent vasomotor regulation of the blood–brain barrier, potentially resulting in abnormal diffusion and transport of metabolites between the interstitial fluid and cerebrospinal fluid. This disruption could, in turn, impair the clearance of amyloid-β from the brain (Ramos-Rodriguez et al., 2013). Additionally, tau may influence endocytosis at the synaptic cleft either directly or indirectly. In the presynaptic terminal, tau has been shown to reduce the mobility of synaptic vesicles at the neuromuscular junction in Drosophila melanogaster, thereby impairing the release of neurotransmitters (Zhou et al., 2017). Specifically, 2N4R tau oligomers decrease presynaptic density and disrupt neuronal trafficking. Tau also plays a role in regulating neuronal excitability, and its effects can vary depending on the biological model. Under pathological conditions, reducing tau levels has been found to alleviate network hyperexcitability in both mouse and fly models (Holth et al., 2013; Lasagna-Reeves et al., 2011).

Due to these reasons, it has been observed that acetylcholine levels are reduced in the brains of AD patients. Therefore, some drugs used in the cholinergic pathway such as cholinesterase inhibitors in the treatment of AD aim to increase the amount of acetylcholine in the brain by preventing the hydrolysis of acetylcholine (e.g. Donepezil, Rivastigmine, Galantamine). As a member of the carboxylesterase enzyme family, AChE mediates the breakdown of acetylcholine (ACh) into acetic acid and choline. Within neural circuits, its primary function involves hydrolysing residual ACh that persists in synaptic clefts following neurotransmission. The cholinergic hypothesis refers to the selective decreased level of ACh in the brain caused by increased activity of AChE in AD and old age (Gauthier et al., 2005; Holzgrabe et al., 2007). In the post-mortem brains of patients with AD, it was associated between diminishing activity of the choline acetyltransferase which syntheses acetylcholine and increasing numbers of neurotic plaques (Braak & Del Tredici, 2012; Mesulam, 2013).

**Cholinesterase inhibitors used in cholinergic pathway**

At present, approved treatments for AD include cholinesterase inhibitors, N-methyl-D-aspartate (NMDA) receptor antagonists, and their combination. Current therapeutic approaches to AD predominantly aim to restore cholinergic neurotransmission by employing agents that inhibit the enzymatic degradation of ACh (Massoud & Gauthier, 2010; PERRY et al., 1978). Cholinesterase inhibitors are specifically developed to prevent the breakdown of ACh, thereby enhancing and prolonging its action at cholinergic synapses. The cholinesterase inhibitors currently approved by the Food and Drug Administration (FDA) for the treatment of AD are donepezil, tacrine, rivastigmine, and galantamine.

A) Donepezil is a highly selective, reversible cholinesterase inhibitor with both non-competitive and competitive effects. It is mainly metabolized by CYP3A4 and CYP2D6, with elimination primarily through the kidneys. Its long half-life of about 70 hours allows for once-daily dosing. Donepezil is widely used to treat Alzheimer's disease and has been shown to improve cognitive functions such as memory, thinking, language, and behaviour. However, it does not halt or reverse disease progression (Dooley & Lamb, 2000).

B) Rivastigmine is a reversible, moderately strong, non-competitive cholinesterase inhibitor that preferentially targets the G1 isoform of AChE. It is rapidly excreted through the kidneys following metabolism. Significant drug interactions are rare, as rivastigmine shows low plasma protein binding and is mainly broken down by esterases rather than liver enzymes (Spencer & Noble, 1998).

C) Galantamine is a reversible and selective AChE inhibitor. Additionally, it binds to a distinct allosteric site on the α-subunit of nicotinic acetylcholine receptors, inducing conformational changes that enhance receptor activity. This provides an added mechanism of action. Galantamine is primarily metabolized by cytochrome P450 enzymes, especially CYP2D6 and CYP3A4. Galantamine and other drugs have been identified that can be used to alleviate the symptoms of the disease (Maelicke et al., 2001; Scott & Goa, 2000).

D) Tacrine was the first approved drug for mild to moderate AD. It acts as a reversible, noncompetitive allosteric inhibitor of AChE. However, its use was limited due to liver toxicity. Tacrine is extensively metabolized by the CYP1A2 enzyme, and its elimination half-life is about 4 hours in AD (Summers, 2000). These drugs have been shown in studies to delay the progression of cognitive and behavioural dysfunctions that are symptoms of AD patients (Massoud & Gauthier, 2010). Longitudinal investigations have demonstrated that donepezil, a cholinesterase inhibitor, correlates with significantly attenuated cortical thinning and reduced basal forebrain degeneration in AD patients (Hampel et al., 2019; Massoud & Gauthier, 2010). Notably, perfusion MRI studies revealed significant cerebral blood flow improvements in prefrontal and limbic areas after prolonged (18-month) treatment with either donepezil or galantamine. This vascular benefit aligns with aggregated clinical evidence from 26 studies confirming that these cholinesterase inhibitors produce clinically relevant stabilization of cognitive functions, behavioural manifestations, and global disease progression in AD patients (Hansen et al., 2008).

**2- The amyloid beta (Aβ) and tau-protein aggregation hypotheses**

**The role of Aβ protein in AD**

The signalling pathway leading to amyloid beta (Aβ) production starts with the cleavage of amyloid precursor protein (APP), a 770-amino acid protein. Aβ having various cellular roles such as synaptic development, copper regulation, maintenance of sphingomyelin and cholesterol balance, neurogenesis, angiogenesis, repairing damage to the blood-brain barrier, develop ability of learning and memory and support of extracellular matrix formation, and acting as an antimicrobial peptide and a tumour suppressor (Müller et al., 2017). In the amyloidogenic pathway, APP is sequentially cleaved by β-secretase and γ-secretase, resulting in the production of Aβ peptides. In contrast, in the non-amyloidogenic pathway, APP is cleaved by α-secretase and γ-secretase, which prevents the formation of Aβ peptides. Aβ is cleaved sequentially by APP beta-domain amyloid precursor protein cleavage enzyme 1 (BACE1), alpha secretase

and γ-secretase to produce the Aβ intracellular domain (AICD) and Aβ proteins of different amino acid lengths (39-41 range) (Flood et al., 1996; Morley & Farr, 2012). Among the Aβ isoforms formed, the most abundant is Aβ1-40 protein with approximately 90%, while Aβ1-42 accounts for approximately 5%. The rest consists of other isoforms. Aβ1-40 plays a role in the regulation of various functions of the cell in normal physiological processes. Increased production of Aβ1-42 is a common feature of AD, and increased aggregation of this peptide is believed to be responsible for driving neurotoxicity (Morley & Farr, 2012; Pettit et al., 2001). Aβ exhibits different behaviours depending on the dose. When synthesized physiologically at picomolar levels, it increases the level of learning and cognition, but as the dose increases, it causes memory loss and learning difficulties (Müller et al., 2017). Specifically, dose-related situation called as "hormesis" for memory so, a lower dose is stimulatory while higher doses are inhibitory. This situation is validated with assays for a lot of hormones such as histamine, acetylcholine, serotonin, gamma amino butyric acid, norepinephrine, dopamine and the NMDA receptor (Farr et al., 1999; Morley & Farr, 2012). Aβ has been shown to stimulate nicotinic ACh receptors, leading to elevated ACh levels in the hippocampus (Pettit et al., 2001). At low concentrations, amyloid-beta also enhances presynaptic activity, promotes dendrite-like branching, supports neurite extension, and improves neuronal survival (Quintanilla et al., 2012; Tillement et al., 2011). Although Aβ exhibits neurogenic potential, it is predominantly viewed as an inhibitor of synaptogenesis. Elevated levels of oligomeric Aβ have been shown to induce dendritic spine retraction and contribute to synaptic degeneration. Synapse loss has been linked to increased intracellular APP expression, accumulation of Aβ within neurons, and the presence of extracellular Aβ near amyloid plaques. Nonetheless, chronic exposure to Aβ at picomolar concentrations has been reported to enhance dendritic spine density in brain slice cultures. In addition, deletion of APP in hippocampal neurons results in diminished synaptic branching and connectivity, and APP-deficient mice exhibit substantial reductions in synaptic protein level. These effects potentially attributed to the absence of sAPPα, a neuroprotective cleavage product of APP (Kent et al., 2020). The hormetic potential of Aβ implies that its lifelong physiological production, driven by learning processes, may ultimately result in toxic accumulation, especially under conditions of impaired clearance mechanisms. Furthermore, it has been reported that moderate levels of APP expression in rat cortical neurons reduced cholesterol synthesis and disrupted cholesterol balance, whereas reduced APP expression had the opposite effect (Pierrot et al., 2013). More recently, it was identified developmental abnormalities in APP-knockout human induced pluripotent stem cells (iPSCs) during their differentiation into neurons; however,

these impairments were reversed by enhancing cholesterol availability and membrane cholesterol levels (Mesa et al., 2024). It has suggested that C99, a membrane-bound fragment generated by BACE1 cleavage of APP, functions as a lipid-sensing molecule that facilitates the transport of cholesterol from the plasma membrane to the endoplasmic reticulum, contributing to the formation of lipid raft–like domains (also known as mitochondria associated endoplasmic reticulum membranes) associated with mitochondria (Montesinos et al., 2020). Additionally, BACE1 has been implicated in normal synaptic development, as evidenced by BACE1 knockout mice exhibiting auditory deficits due to disrupted synaptic architecture in the cochlea. Notably, administration of BACE1 inhibitors to adult wild-type mice results in decreased dendritic spine density and impaired spine formation. Conversely, in APP/PS1 transgenic mice, which overproduce Aβ, BACE1 inhibition has been shown to decelerate synaptic degradation in regions surrounding amyloid plaques. These findings suggest the existence of a critical Aβ concentration necessary for synaptic maintenance (Dierich et al., 2019; Peters et al., 2018).

In addition, it is known that Aβ plays a role in the formation of the myelin sheath. Myelin is known as a protective covering around nerve cells in the nervous system. Myelin serves as an insulating sheath surrounding nerve fibers in the central and peripheral nervous systems. Studies in BACE1 knockout (BACE1−/−) mice have demonstrated delayed myelination and decreased myelin sheath thickness in both the CNS and PNS, as well as impaired remyelination following peripheral nerve injury (Hu et al., 2006). These effects appear to be independent of Aβ production and are likely related to disrupted cleavage of neuregulin proteins, suggesting a potential unintended consequence of targeting Aβ via BACE1 inhibition. Interestingly, levels of BACE1, neuregulin-1, and Aβ rise during the remyelination process after ischemic stroke, indicating a possible role in myelin repair. Conversely, oligomeric Aβ1-42 has been shown to decrease oligodendrocyte populations and cause motor dysfunction when administered intracerebroventricularly in mice (Zhang et al., 2018).

Research also indicates that Aβ is involved in vascular development. In vitro findings demonstrate that Aβ suppresses capillary formation by endothelial cells in a concentration-dependent fashion, while high levels of Aβ promote capillary breakdown (Paris et al., 2004). Conversely, at lower concentrations, Aβ has been shown to enhance endothelial cell proliferation, facilitate the formation of capillary-like networks, and increase microvascular density in brain slice models (Durrant et al., 2020). In vivo evidence supports that Aβ peptides can elevate capillary density and promote the emergence of sprouting endothelial tip cells. Furthermore, mice lacking BACE1 exhibit a marked decrease in retinal vascular

density, and both APP-deficient zebrafish and those exposed to BACE1 inhibitors present with shortened hindbrain vasculature and reduced cerebrovascular branching (Luna et al., 2013). Moreover, overproduction of amyloid beta disrupts the transport system of essential proteins and amyloid beta into the mitochondria of neurons. Proteins accumulated in the endoplasmic reticulum trigger inflammation and cause disruption of the function of mitochondria (Tillement et al., 2011). The function of the mitochondrial electron transport system's complex I enzyme is disrupted and an increase in the amount of reactive oxygen species is observed, and oxidative damage develops (Quintanilla et al., 2012). Therefore, AD patients experience a decreased energy metabolism. Oxidative damage causes hyperphosphorylation of tau proteins and disruption of the mitochondria system. Oxidative stress, moreover, increases the activity of Beta secretase enzyme which also increases amyloid beta production. These lead to loss of neuronal communication and development of AD (Reddy, 2011). Furthermore, amyloid-beta efflux across the blood–brain barrier is regulated by lipoprotein receptor-related protein 1 (LRP-1). Inactivation of LRP-1 in young mice led to increased brain amyloid-beta accumulation and cognitive impairment (Jaeger et al., 2009). It is reported that although no reduction in LRP-1 levels was observed in brains with AD, an elevation of the lipid peroxidation product 4-hydroxy-2-nonenal, which binds to transmembrane LRP-1, was detected in the hippocampus of individuals with AD (Owen et al., 2010). This interaction is thought to contribute to amyloid-beta accumulation. Therefore, it also may be contributed to the progression of AD.

**The role of Tau protein in AD**

Tau is a hydrophilic, axonal protein belonging to the Microtubule-Associated Protein (MAP) family. Tau protein is one of the primary microtubule-associated proteins (MAPs) found in neurons. Its principal function is to stabilize and promote the assembly of microtubules. Microtubules, as components of the cytoskeleton, are essential for maintaining cell shape, enabling intracellular transport, and supporting processes such as cell division and movement. Encoded by the MAPT gene on chromosome 17q21.31, tau contains 16 exons (Parra Bravo et al., 2024). Structurally, it consists of four key domains: an N-terminal domain, a proline-rich region (PRR), a microtubule-binding region (MTBR), and a C-terminal domain. The MTBR harbours two critical hexapeptide motifs, VQIINK and VQIVYK, which facilitate tau's propensity to form β-sheet structures, ultimately promoting pathological aggregation (Von Bergen et al., 2000). While the N terminus is negatively charged, the PRR region is positively charged at pH 7, and the signalling protein SH3 binds to the PRR region (Bachmann et al., 2021). Moreover, C terminal has mixed

charged amino acid motifs that support with microtubule assembly. Tau protein expressed in neurons enables the assembly of microtubules and stabilizes microtubule networks. Soluble monomeric tau plays a role in maintaining genomic integrity by stabilizing DNA, regulating nuclear calcium signalling, and influencing chromatin organization (Parra Bravo et al., 2024). It also contributes to the stabilization of axonal microtubules, thereby supporting microtubule polymerization and axonal projection dynamics. Additionally, at the axon initial segment, tau helps form a diffusion barrier that regulates protein trafficking into the axon and ensures the compartment's functional integrity (Aronov et al., 2001; Thies & Mandelkow, 2007). Different 4 microtubule domains (R1-R4) are formed with the addition of different exons during transcription modification (Chen et al., 2011). Of these domains, 3R is the innate stable domain. In a healthy adult human brain, 3R and 4R isoforms of tau are expressed in close ratios, but this ratio changes in neurodegenerative diseases (Hutton et al., 1998). In AD, both isoforms are expressed but their ratios change during disease progression (Arai et al., 2003). Tau protein can undergo phosphorylation at more than 80 sites, especially within PRR and MTBR. Phosphorylation at residues such as threonine 231 and serine 262 is known to weaken tau's binding to microtubules (Sengupta et al., 1998). Under normal physiological conditions, kinases and phosphatases tightly regulate tau phosphorylation, typically resulting in two to three phosphate groups per tau molecule. However, excessive phosphorylation increases tau's tendency to aggregate (Köpke et al., 1993). This hyperphosphorylated form of tau often becomes mis-localized to dendritic spines, interfering with synaptic function. In AD having brains, phosphorylated tau has been found to associate with proteins involved in synaptic regulation and phagosome-related mechanisms (Drummond et al., 2020). Hyperphosphorylated tau polymerizes into insoluble intracellular filaments, forming neurofibrillary tangles (NFTs). These aggregates are pathognomonic for tauopathies such as frontotemporal lobar degeneration, progressive supranuclear palsy, AD, and chronic traumatic encephalopathy (Götz et al., 2019; Parra Bravo et al., 2024). Although amyloid plaques appear years before mild cognitive impairment, it is the buildup of tau inclusions that more closely aligns with cognitive deterioration, suggesting tau plays a key role in advancing the disease (Bejanin et al., 2017). To clearance of excess tau or dysfunctional tau, ubiquitylation is used to regulate tau clearance by the ubiquitin-proteasome and autophagy systems. Three E3 ubiquitin ligases are involved in tau clearance, particularly in regions rich in lysine amino acid residues (Kim et al., 2021). Endogenous tau protein samples from mice have been shown to be ubiquitylated at specific sites in the MTBR, and similarly, ubiquitylation of specific sites in the MTBR has been shown in humans (Morris et al., 2015). Moreover, abnormal acetyl-

ation may interfere with ubiquitination at identical lysine sites, thereby impairing tau clearance and contributing to the build-up and propagation of disease-associated tau species, including its phosphorylated forms (Min et al., 2010). Proteomic analyses identify substantial overlap between acetylation and ubiquitination sites in tau, particularly within the PRR and MTBR domains, in both experimental AD models and human neuropathological samples. Hyperacetylated tau species have been identified in brain lysates of individuals with AD to cause NFTs (Wesseling et al., 2020). Additionally, tau acetylation promotes its movement into the nucleus, while mutations that simulate hyperphosphorylation-related toxicity trigger nuclear accumulation of tau, ultimately triggering cell death. Therefore, disruption of the ubiquitination and acetylation systems could lead to tau aggregation and contribute to the development of AD. However, it is not yet known why the ubiquitination and acetylation system is disrupted. Mutations in the responsible genes in this system may be one of the reasons.

Moreover, autophagy and the ubiquitin-proteasome system (UPS) play critical roles in the degradation and recycling of cellular macromolecules. Under normal physiological conditions, misfolded tau proteins are typically eliminated through these pathways (Li et al., 2022). However, in AD, tau aggregates progressively accumulate. While tau ubiquitylation facilitates its degradation via the UPS, it can also increase tau's insolubility, a phenomenon observed during the advanced stages of neurofibrillary tangle (NFT) formation (Piras et al., 2016). The autophagy–lysosomal pathway facilitates the degradation of tau fragments and the clearance of tau inclusions. However, suppression of macro autophagy, such as through dysregulation of the mechanistic target of rapamycin (mTOR) pathway, can exacerbate tau aggregation and toxicity (Wang et al., 2010). Acetylated tau inhibits chaperone-mediated autophagy (CMA), thereby promoting the spread of tau. Conversely, activation of CMA in tau-transgenic AD models has been shown to mitigate tau pathology and enhance cognitive performance (Bourdenx et al., 2021). Furthermore, mitophagy, the targeted autophagic removal of damaged mitochondria, is essential for maintaining mitochondrial health. Under conditions of cellular stress or injury, the kinase PINK1 and the E3 ubiquitin ligase tag dysfunctional mitochondria through phosphorylation-dependent ubiquitination at Ser65, marking them for degradation (Hou et al., 2021). In AD brains, elevated Ser65 ubiquitination has been linked to granulovacuolar degeneration and the early development of phosphorylated tau aggregates. Enhancing mitophagy through B vitamin (NAD) supplementation has been shown to decrease tau hyperphosphorylation and attenuate neuroinflammatory responses (Hou et al., 2018).

Although drug development efforts targeting tau protein are ongoing, they have yet to yield successful outcomes. Current approaches as follows immunotherapies directed at phosphorylated tau or specific amino acid residues within the microtubule-binding region of the tau protein, antisense oligonucleotides aimed at the MAPT gene, which encodes tau, and small-molecule inhibitors targeting O-GlcNAcase which is the enzyme responsible for removing N-acetylglucosamine modifications from tau.

**3- The role of metal ions in the pathogenesis and progression of AD**

Zinc, iron, and copper play essential roles in various physiological processes, such as enzymatic catalysis, stabilization of protein structures, cellular signalling, and metabolic regulation. However, the recent studies reported that accumulating evidence has pointed toward a crucial role of metal ion dyshomeostasis in the etiology and progression of AD, particularly involving iron (Fe), copper (Cu), zinc (Zn), and calcium (Ca) (Devos et al., 2014).

**Iron and ferroptosis in AD**

Iron is the most prevalent transition metal in the human body. Iron is a redox-active transition metal vital for numerous physiological functions, including oxygen transport, DNA synthesis, and mitochondrial respiration (Huang et al., 2004). In the brain, its concentration averages approximately 0.04 mg per gram of fresh tissue, corresponding to around 720 μM. The highest iron levels are found within the extrapyramidal system, particularly in the basal ganglia, whereas comparatively lower levels are observed in the cerebral cortex, and the lowest concentrations are noted in the white matter and medulla oblongata (Morris et al., 1992). Neuronal uptake of iron occurs primarily through transferrin (Tf), divalent metal transporter 1 (DMT1), and lactoferrin (Lf), while ferroportin (FPN) mediates its efflux. Lactoferrin, a multifunctional iron-binding glycoprotein, exhibits a strong affinity for ferric ions ($Fe^{3+}$). Transferrin-bound iron is distributed systemically via the circulatory system. Consequently, disruptions in the expression or function of DMT1, Tf, Lf, and FPN may lead to imbalances in iron homeostasis, contributing to abnormal iron accumulation in the brain. Iron accumulates in brain regions vulnerable in AD, such as the hippocampus and cortex (van Duijn et al., 2017). Additionally, under physiological conditions, tau facilitates the trafficking of APP to the neuronal membrane, where APP interacts with and stabilizes ferroportin, thereby promoting the export of ferrous iron ($Fe^{2+}$) from neurons. A decrease in soluble tau or the presence of familial AD (FAD)-associated APP mutations disrupts this process, leading to impaired iron efflux and intracellular iron accumulation. Moreover, cleavage of APP by BACE1

hinders its ability to stabilize ferroportin on the cell surface, further contributing to iron retention within neurons (Tsatsanis et al., 2019).

Moreover, Heme oxygenase-1 (HO-1) enzyme plays an important role iron accumulation in brain with AD. HO-1 is an enzyme that helps break down heme group of hemoglobin into $Fe^{2+}$, carbon monoxide, and biliverdin. Although HO-1 activity can reduce oxidative stress by removing harmful heme, too much HO-1 can increase oxidative damage and lead to ferroptosis by releasing excess $Fe^{2+}$ into the cell. In AD, HO-1 levels are often found to be higher in astrocytes in affected brain regions, but lower in blood plasma and cerebrospinal fluid (Schipper et al., 2019). Iron's interaction with amyloid-beta (Aβ) and tau protein exacerbates neuropathology by promoting Aβ aggregation and tau hyperphosphorylation, facilitating the formation of senile plaques and neurofibrillary tangles, and enhancing their neurotoxic potential. Ferrous ions ($Fe^{2+}$), when bound to the N-terminal domain of Aβ, can induce structural modifications and catalyse the production of reactive oxygen species, damaging neuronal membranes (Wärmländer et al., 2019). Furthermore, in SH-SY5Y human neuroblastoma cells, elevated ferric iron ($Fe^{3+}$) has been shown to bind to APP mRNA, enhancing its translation and activating β-secretase, thereby increasing Aβ synthesis (Banerjee et al., 2014). Moreover, iron-induced lipid peroxidation damages neuronal membranes, disrupts synaptic signalling, and leads to neurodegeneration. Post-mortem analysis has revealed iron deposits within Aβ plaques and NFTs, suggesting a direct contribution to pathology (Ke & Qian, 2003).

Iron chelation therapy, using agents such as deferoxamine, has demonstrated neuroprotective effects in experimental AD models by reducing oxidative stress and delaying cognitive decline. These findings underline the therapeutic potential of targeting iron overload and ferroptosis in AD treatment strategies.

**The role of copper in AD**

Copper (Cu), a redox-active trace metal, is essential for neuronal function, participating in processes such as neurotransmission, antioxidant defence, and enzymatic catalysis. However, disruption of copper homeostasis has been closely associated with the pathogenesis of Alzheimer's disease (AD). In healthy neurons, $Cu^{2+}$ is imported via copper transporter 1 (CTR1) and exported by ATP7A and ATP7B (Curnock & Cullen, 2020). Studies indicate that, while bulk Cu levels are often decreased in AD brains (Chaudhari et al., 2023; Xu et al., 2017), there is a concomitant increase in labile, cytoplasmic $Cu^{+}$ ions. This imbalance alters the redox environment and may activate tau kinases such as CDK5 and GSK3, pro-

moting tau hyperphosphorylation (Lei et al., 2021). Cu plays a significant role in amyloid pathology. It binds with high affinity to Aβ peptides, particularly at their histidine residues, facilitating the formation of redox-active Cu–Aβ complexes. These complexes generate hydrogen peroxide ($H_2O_2$), promoting oxidative stress and membrane lipid damage (Curtain et al., 2003; Rongioletti et al., 2018). In vitro studies have also demonstrated that Cu enhances Aβ aggregation and can induce tau aggregation via similar redox mechanisms (Xu et al., 2017). The genetic regulation of Cu transport further links copper dysregulation to AD. Mutations in ATP7B, a gene known for its role in Wilson's disease, have been shown to selectively impair Cu efflux in AD patients, but not in other dementia subtypes, indicating a disease-specific vulnerability (Rongioletti et al., 2018). ATP7B dysfunction increases intracellular $Cu^+$ accumulation and may exacerbate tau-related neurodegeneration. During synaptic activity, transient Cu release from synaptic vesicles modulates excitatory transmission by acting as a potent inhibitor of NMDA and AMPA receptors. Cu can also influence GABAergic and purinergic signalling, in addition to altering vesicular trafficking and synaptic protein interactions (Wang et al., 2020). From a therapeutic perspective, Cu chelators have shown potential in preclinical models. Chelation has been found to reverse Aβ aggregation and reduce oxidative stress (Ejaz et al., 2020). Transgenic AD mouse models treated with chelators displayed improved cognition and reduced neurotoxicity. However, clinical results have been mixed. A phase II clinical trial using copper orotate supplementation did not yield significant benefits in AD patients. Moreover, excessive Cu supplementation in AD models resulted in increased tau phosphorylation and cognitive deficits (Chen et al., 2019).

Thus, while copper is essential for brain function, its mis-regulation contributes to both amyloid and tau pathology in AD. Future therapies must balance Cu's physiological roles with its pathological potential.

**The role of zinc in AD**

Zinc (Zn) is a vital trace element in the central nervous system, serving critical roles in enzymatic catalysis, structural stabilization of proteins, and synaptic transmission. Approximately 80–90% of Zn in the brain is protein-bound, supporting cellular structure and function, and over 2,800 Zn-binding proteins have been identified in the human proteome (Paoletti et al., 2009). A smaller pool of Zn is stored in glutamatergic synaptic vesicles at concentrations exceeding 100 μM, where it is co-released with neurotransmitters during synaptic activity, modulating signal transmission and contributing to synaptic plasticity (Datki et al., 2020). Synaptic Zn turnover is essential for cognitive maintenance and plasticity, especial-

ly in the hippocampus where Zn modulates long-term potentiation (LTP). However, this regulatory mechanism becomes impaired with aging, which may underlie age-related cognitive decline and contribute to AD pathogenesis (Datki et al., 2020). Metallothionein 3 (MT3), a Zn-binding protein predominantly expressed in the brain, plays a pivotal role in buffering free $Zn^{2+}$. MT3 sequesters Zn as metal-thiolate clusters and can exchange Zn with Cu in Cu–Aβ complexes, thus mitigating oxidative stress (Wang et al., 2020). In AD, MT3 expression is downregulated, resulting in increased vulnerability to oxidative damage and impaired Zn homeostasis. Under pathological conditions, excessive Zn release from neurons and astrocytes activates NADPH oxidase, enhancing reactive oxygen species (ROS) production and triggering microglial activation and neuronal apoptosis (Furuta et al., 2016). Zn accumulation within mitochondria disrupts the respiratory chain and further elevates oxidative stress. Moreover, Zn overload can enhance amyloid-β (Aβ) production and aggregation. The nature of Zn-induced Aβ aggregates depends on the Zn/Aβ ratio—stoichiometric levels favor the formation of soluble, non-fibrillar oligomers, while substoichiometric Zn levels promote β-sheet-rich fibrils, which are more neurotoxic. Zinc concentrations in amyloid plaques can reach millimolar levels. Imaging techniques such as X-ray fluorescence microscopy and metallomic mass spectrometry have confirmed elevated Zn levels in Aβ plaques of APP/PS1 and Tg2576 transgenic mice, as well as in aged primate models (Lei et al., 2021). Zn transport across neuronal membranes is regulated by ZIP (Zrt-Irt-like proteins) importers and ZnT (zinc transporter) exporters. ZnT3, localized to glutamatergic synaptic vesicles, is particularly relevant to AD. ZnT3 expression declines with age, disrupting synaptic Zn release and recycling. Conversely, in conditions of oestrogen deficiency, such as menopause, ZnT3 levels may rise, increasing extracellular $Zn^{2+}$, which binds to Aβ and fosters plaque formation (Lei et al., 2021; Xu et al., 2019). Increased cytosolic $Zn^{2+}$ levels may also exacerbate tau pathology. Zn activates kinases such as CDK5, GSK3, ERK1/2, and JNK, while inhibiting phosphatase PP2A, collectively promoting tau hyperphosphorylation (Lei et al., 2021). Moreover, postmortem studies of AD brains report altered expression of multiple ZnTs. ZnT10 is significantly downregulated in the frontal cortex of AD patients and APP/PS1 mice (Xu et al., 2019), while ZnT6 levels are elevated, and ZnT1 levels are decreased in the hippocampus and parahippocampal regions. Zn also interacts with other AD-relevant proteins. It modulates the activity of presenilin 1 and affects the stability of apolipoprotein E (ApoE), particularly the pathogenic ApoE4 isoform (Uddin et al., 2019). Zinc homeostasis is tightly coupled to Aβ generation via its influence on secretase enzymes. For example, α-secretase, a disintegrin and metalloproteinase 10 (ADAM10), requires Zn for its proteolytic ac-

tivity. Disruption of its Zn-binding domain impairs non-amyloidogenic APP cleavage. Moreover, Zn binding may influence APP-C99 dimerization and modulate β-secretase activity, thereby reducing Aβ production (Gerber et al., 2017).

Taken together, the multifaceted role of Zn in AD encompasses disrupted synaptic signalling, increased oxidative stress, enhanced Aβ aggregation, and tau hyperphosphorylation. These findings support the notion that targeting Zn homeostasis may offer therapeutic value in modifying AD progression.

**Use of chelators in AD treatment**

Chelation therapy has been proposed as a strategy to mitigate abnormal accumulations of essential metals such as Fe, Cu, and Zn, as well as toxic metals like lead (Pb), mercury (Hg), and cadmium (Cd). Metal chelators operate by binding to metal ions, thereby limiting their redox activity and impeding their role in amyloid-β (Aβ) aggregation (Chaves et al., 2021). These multifunctional agents not only decrease the intracellular concentration of redox-active metals but also protect against oxidative damage and facilitate Aβ solubilization. Among FDA-approved iron chelators, deferoxamine has demonstrated cognitive benefits in animal models by attenuating amyloidogenic processing of APP and preventing Aβ aggregation (McLachlan et al., 1991). It also suppresses APP translation through interaction with the iron-responsive element in the 5′ UTR of APP mRNA and has shown cognitive improvement in AD patients upon intramuscular administration (Rogers et al., 2002). Deferiprone, a lipid-soluble agent capable of crossing the blood-brain barrier, has effectively reduced cerebral iron accumulation in neurodegenerative disorders in clinical trials (Klopstock et al., 2019). Similarly, deferasirox, which promotes renal iron excretion, has been shown to prevent age-related iron accumulation and oxidative stress markers in the brains of aged rats. Cu and Zn levels can also be modulated by chelators such as penicillamine and trientine, which promote urinary excretion, and tetrathiomolybdate, which enhances biliary clearance of Cu. Other compounds, such as clioquinol and PBT2, target Cu-Aβ and Zn-Aβ complexes. These ionophores facilitate the uptake and redistribution of $Cu^{2+}$ and $Zn^{2+}$, reversing synaptic metal imbalances and improving cognitive function in animal models. PBT2 disrupts metal-Aβ interactions, promoting aggregate clearance and modulating synaptic metal levels by restoring metal ion homeostasis (Banerjee et al., 2015).

Although these agents exhibit neuroprotective effects in preclinical settings, clinical trials have not consistently demonstrated significant efficacy in treating AD, suggesting a need for further optimization of met-

al-targeting strategies.

**4- Oxidative stress role in AD**

Harman's free radical theory of aging is particularly applicable to the central nervous system, which, due to its high oxygen demand and metabolic rate, is highly susceptible to oxidative stress. Neurons are especially vulnerable because they are post-mitotic and cannot be replaced once damaged, making them prone to mitochondrial dysfunction over time (Bonda et al., 2014). In addition, mitochondria located at presynaptic terminals experience repeated calcium influx through voltage-gated calcium channels, accelerating oxidative injury at synapses (Grimm & Eckert, 2017). As the brain ages, markers of oxidative stress, including disrupted metal homeostasis, DNA lesions, and impaired protein metabolism, become increasingly evident. In AD, there is further accumulation of ROS and redox-active metals such as Cu, Fe, Zn, and Mg, all of which contribute to amyloid beta (Aβ) aggregation and tau hyperphosphorylation (Kim et al., 2018). Mitochondria are a primary source of neuronal ROS due to byproducts generated during oxidative phosphorylation. Under conditions of reduced ATP demand, superoxide is formed through incomplete oxygen reduction in the electron transport chain. Excess ROS not only disrupt redox homeostasis and trigger pro-inflammatory signalling (e.g., IL-1, IL-6, TNF-α), but also lead to further mitochondrial impairment, reinforcing oxidative stress in a harmful feedback loop (Islam, 2017; Sinha et al., 2013). Mitochondrial DNA (mtDNA), which lacks protective histones, is particularly susceptible to oxidative damage, and this damage undermines neuronal function. Consequently, mitophagy (a process that removes dysfunctional mitochondria) is essential for neuronal health (Islam, 2017). Although oxidative stress can trigger mitophagy by reducing mitochondrial membrane potential, it can also interfere with key mitophagy regulators, disrupting mitochondrial quality control. This interplay underlies a broader theory that integrates ROS production, DNA damage, and mitochondrial dysfunction in aging. According to this model, DNA damage activates enzymes such as PARP and various kinases, depleting NAD+ that is a cofactor crucial for ATP synthesis and metabolic regulation (Guillaumet-Adkins et al., 2017; Sinha et al., 2013). As NAD+ levels fall, the cell increases mitochondrial respiration to meet energy demands, leading to greater membrane potential, impaired mitophagy, and elevated ROS, which in turn perpetuate DNA damage (Guillaumet-Adkins et al., 2017). This cycle highlights mitochondria as central players in the aging process and in the development of neurodegenerative diseases. Mitochondrial dysfunction is also a hallmark of AD. Increased oxidative modification of mtDNA is one of the earliest observable changes in the AD brain. The mitochondrial cascade hypothesis suggests that age-related

mitochondrial decline affects amyloid precursor protein (APP) processing, leading to the accumulation of toxic Aβ oligomers and subsequent plaque formation (Cheignon et al., 2018).

Moreover, Aβ itself acts as a potent inducer of oxidative damage. Neuronal exposure to Aβ1-42 oligomers results in lipid peroxidation, evidenced by the accumulation of 4-hydroxy-2-trans-nonenal (HNE) bound to proteins (Mark et al., 1997). Due to its hydrophobic structure, Aβ embeds in neuronal membranes, where HNE adducts compromise cellular function and trigger apoptosis. Aβ-mediated oxidative damage also impairs glucose and glutamate transporters, reduces mitochondrial membrane potential, and inhibits Na+/K+ ATPase activity (Butterfield, 2020). These harmful effects are intensified by Aβ's ability to bind redox-active metals, which further drives aggregation and oxidative toxicity. Oxidative stress induced by metal–amyloid beta complexes contribute to excitotoxicity, disrupts membrane potential, and compromises mitochondrial integrity. One of the major consequences of oxidative stress is DNA damage, characterized by apurinic/apyrimidinic sites, oxidized bases, and DNA strand breaks. These breaks are categorized as single-strand breaks (SSBs) and double-strand breaks (DSBs), with DSBs being more deleterious. SSBs result from ROS-mediated degradation of the sugar-phosphate backbone, while DSBs are more persistent and may alter the transcription of genes near break sites (Shanbhag et al., 2019). Elevated DNA damage is observed in both normal aging and AD, with some suggesting that the failure to repair such lesions is a fundamental driver of aging (Maynard et al., 2015). Reduced expression of base excision repair (BER) genes in aging may account for increased SSB accumulation. This increase has been reported in hippocampal pyramidal and granule neurons, and cerebellar granule cells, but not in Purkinje cells (Rutten et al., 2007). Notably, mitochondrial DNA (mtDNA) damage is significantly higher than nuclear DNA damage-up to tenfold in individuals over 42 and fifteenfold in those over 70 years of age. Age-related increases in SSBs impair genes involved in synaptic function, learning, and memory, with mtDNA damage particularly affecting pathways linked to neuronal metabolism and survival (Mecocci et al., 2018). Oxidative stress also induces epigenetic changes, notably through the upregulation of SIRT1, which increases levels of histone H3 lysine 9 trimethylation (H3K9me3), a modification associated with transcriptional repression. SIRT1 plays a protective role by promoting mitophagy and autophagy under oxidative conditions (Fang et al., 2017). Emerging data suggest that increased H3K9me3 may be a cellular response to oxidative stress, and pharmacological inhibition of this pathway might help mitigate age-related cognitive decline and AD pathology. In addition to histone methylation, aberrant DNA methylation

at specific CpG sites has been frequently reported in AD brains. Modifications of histone proteins, such as methylation and acetylation, are key regulators of chromatin dynamics and gene expression. Disruption of these modifications, especially involving H3K9 methylation, appears to contribute to cognitive decline. Upregulation of histone methyltransferases like SUV39H1 and G9a may represent a compensatory mechanism in response to oxidative stress (Behl et al., 2024).

Given the involvement of oxidative damage in AD, therapeutic strategies increasingly focus on limiting oxidative stress through antioxidant compounds. Nutritional supplements such as glutathione, various vitamins, polyphenols, and flavonoids are being explored for their potential to counteract oxidative pathology in AD.

## 5- The role of genetic factors in AD

### APP gene mutations in AD

AD is broadly categorized into early-onset and late-onset forms. Approximately 90% of cases are sporadic, with late-onset AD typically manifesting after the age of 60–65 (Campion et al., 1999). Early-onset AD appears between 30 and 65 years of age, with about 13% of familial cases showing autosomal dominant inheritance patterns. Mutations in the APP, PSEN1, and PSEN2 genes are associated with early-onset familial AD, while the APOE gene remains the most prominent genetic risk factor linked to late-onset AD (Krishnamurthy et al., 2024; Selkoe, 2001). Despite the strong association between the APOE ε4 allele and AD risk, many carriers remain cognitively intact well into old age, indicating the influence of additional genetic and environmental factors in late-onset AD susceptibility. Recent studies have identified several novel genes associated with increased risk for sporadic AD. To date, 69 distinct missense mutations have been reported in the APP gene, accounting for approximately 10–15% of familial early-onset AD cases. These mutations are generally located near the Aβ domain and impact APP cleavage, often resulting in elevated Aβ42 production (Walker et al., 2005). Notable examples include the Swedish mutations (APPSW, M67IL, APPK670N) and the London mutation (APPV717I), which have been shown to promote amyloidogenic processing and facilitate disease development (Kwok et al., 2000). Many of these pathogenic variants occur close to the γ-secretase cleavage site, affecting the ratio and aggregation propensity of Aβ peptides.

### Presenilin 1 (PSEN1) gene mutations in AD

The PSEN1 gene, located on chromosome 14, encodes a core component of the γ-secretase complex, essential for the proteolytic cleavage of

type I transmembrane proteins such as amyloid precursor protein (APP) (Theuns et al., 2000). The PSEN1 protein plays a critical role in stabilizing and activating the γ-secretase complex. Missense mutations in PSEN1 account for approximately 18% to 50% of autosomal dominant early-onset familial AD. These mutations typically increase the ratio of amyloid-beta 42 (Aβ42) to amyloid-beta 40 (Aβ40), favoring the formation of the more aggregation-prone Aβ42 peptide. Experimental models have demonstrated a direct correlation between PSEN1 and APP mutations and elevated Aβ production (Lippa et al., 1998; Wolfe, 2007). Notably, specific PSEN1 mutations, such as N135S, are associated with impaired Notch signalling and present clinically with symptoms including dementia, parkinsonism, limb spasticity, seizures, and altered generation of the amyloid-beta intracellular domain (Rudzinski et al., 2008). To date, over 300 mutations have been identified in PSEN1, the majority of which are missense variants leading to amino acid substitutions distributed throughout the protein. These changes typically elevate Aβ42 production while reducing Aβ40 levels, thereby increasing the Aβ42/Aβ40 ratio and promoting Aβ42 accumulation in the brain (Moehlmann et al., 2002).

**Apolipoprotein E (APOE) gene alleles in AD**

The APOE gene, located on chromosome 19, encodes apolipoprotein E, a key lipid-transport protein predominantly expressed in the brain (Corder et al., 1993). ApoE is primarily synthesized in astrocytes, but also in microglia and neurons, where it facilitates lipid and cholesterol redistribution by forming lipoprotein particles. These particles are essential for lipid delivery to neurons. The ε4 variant of the APOE gene, located on exon 4, is one of three common alleles including ε2, ε3, and ε4. Among them, ApoE3 is the most prevalent isoform. Although the precise mechanisms by which ApoE contributes to neurodegeneration remain unclear, the ε4 allele has been consistently linked to increased AD risk. It promotes amyloid-β aggregation and tau hyperphosphorylation, both hallmarks of AD pathology (Huang, 2006). Uniquely human in origin, APOE polymorphisms are hypothesized to have evolved in response to dietary shifts (Murphy et al., 2013). Carriers of the ε4 allele exhibit enhanced vulnerability to multiple neurodegenerative features, including α-synuclein pathologies, neuroinflammation, synaptic dysfunction, reduced dendritic spine density, impaired plasticity, and accumulation of Aβ oligomers at synapses, as well as accelerated tau-related degeneration (Krishnamurthy et al., 2024).

Additionally, a few genes including PICALM, ABCA7, CLU, CD33, BIN1, SORL1, CR1, EPHA1, CD2AP, MS4A, and TREM2, have been found in association with AD so far. More recently, single nucleotide

polymorphisms (SNPs) linked to an additional fifteen genes have been discovered. However, the precise roles and mechanistic contributions of many of these genes to AD pathogenesis remain poorly understood (Behl et al., 2024).

**Conclusions**

Alzheimer's disease (AD) is recognized as a complex neurodegenerative disorder influenced by multiple etiological factors, and its prevalence is expected to increase significantly in the coming years. To date, numerous studies involving both AD patients and animal models have aimed to elucidate the disease's etiology and pathology; however, the precise underlying causes remain incompletely understood, and research in this area is ongoing. Among the major contributors to AD pathology are tau proteins, which form neurofibrillary tangles through hyperphosphorylation, and $A\beta_{42}$ peptides, which aggregate into insoluble amyloid plaques. In addition, mutations in APP, Presenilin-1 (PSEN1), and Presenilin-2 (PSEN2), as well as the APOE ε4 allele, are known to play critical roles in familial early-onset AD. Other contributing factors include the accumulation of metals such as Fe, Zn, and Cu in various brain regions, which trigger oxidative stress and further promote Aβ and tau aggregation into plaques and fibrils. These pathological aggregates also impair cholinergic neurotransmission by reducing acetylcholine levels, thereby hindering neuronal communication.

Although no curative treatment for AD has yet been discovered, several therapeutic strategies are currently in use. Cholinesterase inhibitors, which prevent the breakdown of acetylcholine, are the most prescribed drugs and help alleviate symptoms and slow disease progression. Additionally, metal chelators are used to reduce metal ion accumulation, though their efficacy remains limited. Antioxidant therapy, including supplementation with vitamins, glutathione, and flavonoids, is also employed to mitigate oxidative stress and thus slow AD progression.

Despite these efforts, AD is projected to remain a major public health challenge, often referred to as the "modern black plague." Therefore, identifying the precise molecular mechanisms underlying the disease and developing effective therapeutics remain urgent priorities.

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