**Zebrafish (*Danio rerio*) as a Platform for Preclinical Research: A Comprehensive Review**

**Abstract**

Zebrafish (*Danio rerio*) have emerged as a powerful and versatile platform for preclinical research due to their remarkable genetic similarity to humans, rapid development, and amenability to genetic and pharmacological manipulation. This freshwater vertebrate model offers unique advantages including transparent embryos, high fecundity, and conserved physiological pathways, which facilitate in vivo visualization and high-throughput screening. Zebrafish have been extensively employed in investigating diverse biomedical fields such as developmental biology, toxicology, pharmacology, and disease modeling. Their use in studying cardiotoxicity, lipid metabolism disorders, tumorigenesis, renal pathologies, and muscular dystrophies has provided critical insights into disease mechanisms and therapeutic interventions. Furthermore, zebrafish serve as an effective tool for drug discovery and preclinical evaluation of novel pharmacological agents, enabling rapid assessment of efficacy and safety profiles. The ethical benefits, cost-effectiveness, and experimental versatility of zebrafish underscore their growing importance in translational research. This comprehensive review discusses the multifaceted applications of zebrafish in preclinical studies, highlighting their contribution to advancing precision medicine and accelerating drug development pipelines.

**Key words:** Zebrafish, Preclinical research, Model organism, Genetic manipulation, Drug screening, Pharmacological evaluation.

**1. Introduction**

Zebrafish (*Danio rerio*), a tropical freshwater teleost native to South and Southeast Asia, have emerged as one of the most versatile vertebrate models in biomedical and pharmacological research. Their natural habitats slow-moving waters like ponds, rice paddies, and streams have shaped their robust adaptability to laboratory environments (McCluskey *et al*., 2020). Initially classified under *Brachydanio rerio*, these small fish exhibit traits highly conducive to experimental science, including rapid development, external fertilization, and high fecundity. A significant advantage of zebrafish lies in their genetic similarity to humans (Lieschke *et al*., 2007). Approximately 70% of human genes have a zebrafish counterpart, and about 84% of genes associated with human diseases are conserved in their genome (Howe *et al*., 2013). Moreover, over 12,000 genes are shared among zebrafish, mice, and humans, allowing for the modeling of diverse pathological states. This homology enables researchers to employ zebrafish in investigating the genetic basis of various human diseases, including neuropsychiatric disorders such as Parkinson’s disease, schizophrenia, and depression (Patel *et al*., 2018). Beyond genetic resemblance, zebrafish exhibit anatomical and physiological systems parallel to those in humans, such as hematopoietic lineages, a central nervous system, a beating heart, a pancreas, and kidneys. Their transparent embryos further enhance their value in developmental biology, allowing in vivo visualization of organogenesis and gene expression patterns without invasive methods (Teame *et al*., 2019). Their omnivorous diet and capacity for high-throughput breeding make zebrafish particularly well-suited for experimental pharmacology and toxicological assessments. While standardized feeding protocols exist, some variability in laboratory conditions can influence metabolic and reproductive parameters, necessitating careful dietary control. Because of their cost-effectiveness, ease of genetic manipulation (Hwang *et al*., 2013), and ethical acceptability relative to mammalian models, zebrafish have become indispensable in modeling a wide array of human conditions from metabolic and cardiovascular disorders to cancer, infectious diseases, and congenital abnormalities. Their increasing integration into preclinical pipelines underscores their pivotal role in advancing drug discovery, disease modeling, and translational medicine (Patton *et al*., 2021; Kari *et al*., 2007).

**1.1 Taxonomy of Zebrafish**

Zebrafish (*Danio rerio*) are classified taxonomically within the kingdom *Animalia*, phylum *Chordata*, and class *Actinopterygii*, which includes all ray-finned fishes. They belong to the order *Cypriniformes* and the family *Cyprinidae*, a diverse group of freshwater fish known for their ecological and biological significance. The genus *Danio* comprises several small, elongated species, among which *D. rerio* is the most widely studied due to its relevance in biomedical research.

**Taxonomic Hierarchy of Zebrafish:**

**Kingdom:** Animalia

**Phylum:** Chordata

**Class:** Actinopterygii

**Order:** Cypriniformes

**Family:** Cyprinidae

**Genus:** Danio

**Species:** *Danio rerio*

This classification underpins the zebrafish's evolutionary proximity to other vertebrates, contributing to its widespread adoption in genetic, toxicological, and developmental biology studies (McCluskey *et al*., 2020).

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Figure 1. Zebrafish **(***Danio rerio***)**

### ****1.2 Reproductive Characteristics and Life Cycle of Zebrafish****

Zebrafish (Danio rerio) reproduce via external fertilization, wherein females release eggs into the aquatic environment, followed by sperm release from males for fertilization. This species exhibits no parental care post-fertilization, with embryos left to develop independently. Although zebrafish typically attain sexual maturity between 10 to 12 weeks of age, researchers often prefer to commence breeding at around six months to ensure higher embryo viability and improved reproductive performance (Allison *et al*., 2009). Adult zebrafish are relatively small, measuring between 4 and 5 centimeters in length. They possess a torpedo-shaped body adorned with alternating light and dark horizontal stripes. Sexual dimorphism is distinctly observable, especially before spawning events. Males generally appear more vibrant with a golden sheen on their ventral side and a slightly slimmer build, while females tend to exhibit a broader abdomen and silvery belly due to the presence of developing eggs. Females are asynchronous spawners, capable of producing and releasing eggs every 2 to 3 days. A single spawning event can yield up to 200 eggs, often distributed over multiple clutches in a day. Embryonic development is rapid; fertilized eggs hatch within 48 to 72 hours post-fertilization. The resulting larvae, known as fry, grow swiftly and usually reach sexual maturity within 2 to 3 months under optimal laboratory conditions (Poleo *et al*., 2001).

**2. BIOMEDICAL RESEARCH ADVANCEMENTS THROUGH ZEBRAFISH MODELS**

Zebrafish (Danio rerio) have gained prominence in biomedical research owing to their distinct biological and genetic features, offering several advantages over traditional rodent models. Their high fecundity enables the generation of hundreds of embryos per spawning, making them well-suited for large-scale experimental studies (Kalueff *et al*., 2014). The transparency of zebrafish embryos further enhances their utility, allowing real-time, non-invasive visualization of embryogenesis and organ development. This facilitates detailed observation of cellular dynamics, organogenesis, and gene expression without sacrificing the organism (Dooley *et al.,* 2000). The rapid embryonic development—completing within approximately 72 hours provides a time-efficient platform for examining developmental biology and pharmacological effects (Patton *et al*., 2021). Female zebrafish can produce 200–300 fertilized eggs per week, permitting repeated experimental cycles and robust data generation. Moreover, zebrafish are genetically homologous to humans in many critical pathways, particularly those involving the central nervous system and immune regulation, making them valuable models for studying neurological diseases, genetic disorders, and human pathophysiology (Howe *et al*., 2013). Zebrafish are highly amenable to genetic manipulation techniques, such as morpholino-based gene knockdown, CRISPR/Cas9 gene editing, and transgenic line development, enabling targeted analysis of gene function and disease mechanisms. Their small size and minimal husbandry requirements allow for high-density housing, which significantly reduces laboratory costs in comparison to rodent models. Additionally, their lower susceptibility to stress ensures greater consistency in behavioral and physiological experiments, enhancing data reproducibility. These cumulative features underscore the zebrafish’s pivotal role in advancing research across drug discovery, disease modeling, and genetic investigations.

### ****2.1 Zebrafish as a Model for Human and Animal Vaccination Research****

The zebrafish model is increasingly being utilized in vaccine research due to its robust immune system and biological attributes that enable efficient and ethical experimentation. Zebrafish possess both innate and adaptive immune responses, including macrophages, neutrophils, complement proteins, T cells, and B cells paralleling the mammalian immune system (Meijer *et al.,* 2011). The external fertilization and optical transparency of embryos allow researchers to observe immunological responses dynamically and in vivo, making them particularly useful for early-stage immunological investigations (Trede *et al.,* 2004). The high fecundity of zebrafish supports large-scale vaccine screenings, and the embryos’ rapid development enables timely assessment of immunogenicity and safety. Zebrafish are also naturally susceptible to a broad range of pathogens bacteria, viruses, fungi, and protozoa mirroring the microbial challenges faced in human and veterinary contexts. This makes them ideal for assessing pathogen-host interactions, immune evasion mechanisms, and vaccine-mediated protection (Bailone *et al*., 2020). Because of these features, zebrafish offer a high-throughput, cost-effective platform for evaluating novel vaccine formulations, understanding immune system ontogeny, and studying adjuvant mechanisms. Their versatility provides promising translational applications for both human and veterinary immunoprophylaxis (Lam *et al*., 2004).

### ****2.2 Zebrafish as a Model for Cancer Research and Genetic Manipulation****

Zebrafish are extensively used in cancer biology due to their genetic malleability and suitability for modeling tumor development and progression. Forward and reverse genetic approaches in zebrafish have facilitated the identification of oncogenes, tumor suppressor genes, and molecular markers associated with malignancies (Amatruda *et al*., 2002). Transparent embryos and specific strains like casper allow in vivo imaging of tumorigenesis, angiogenesis, and metastasis in real-time (White *et al*., 2008). Transgenic zebrafish lines overexpressing human cancer-related genes (e.g., MYC, KRAS) or bearing mutations in tumor suppressors (e.g., tp53) replicate various human cancer phenotypes including leukemia, melanoma, and hepatocellular carcinoma (Langenau *et al*., 2003). Human cancer cells can also be xenografted into zebrafish embryos, offering a live model to monitor tumor growth and evaluate chemotherapeutic responses. Additionally, their use in high-throughput drug screening has streamlined the discovery of anticancer compounds and potential gene-drug interactions. These advantages have made zebrafish an indispensable model for functional oncology and preclinical therapeutic screening

### ****2.3 Zebrafish as a Model for Epilepsy Research****

Zebrafish provide an effective model for epilepsy research due to their neurological homology with humans and suitability for genetic manipulation. Around 85% of genes linked to epilepsy in humans have homologs in zebrafish (Baraban *et al*., 2013). The ease of introducing targeted mutations allows researchers to investigate gene-specific contributions to seizure susceptibility. Drug administration in zebrafish larvae is highly efficient, as they absorb compounds directly from their aquatic environment. This facilitates rapid testing of antiepileptic agents and neurotoxicity screening. Behavioral and electrophysiological assessments in zebrafish larvae have been validated to model human seizure activity, enabling high-throughput analyses of drug efficacy (Kearney *et al*., 2018). The scalability and genetic transparency of zebrafish render them highly suitable for exploring the molecular underpinnings of epilepsy, identifying therapeutic targets, and testing novel interventions.

### ****2.4 Zebrafish as a Model for Studying Diabetes Mellitus****

Zebrafish are a valuable model for studying metabolic diseases, particularly diabetes mellitus. Their pancreas shares developmental pathways and cellular architecture with mammals, including insulin-producing β-cells, exocrine cells, and glucagon-secreting α-cells (Field *et al*., 2003). External cues like fibroblast growth factor (FGF), retinoic acid, and Sonic Hedgehog (Shh) are known to regulate pancreatic morphogenesis in both zebrafish and humans. Hyperglycemia can be experimentally induced in zebrafish by glucose immersion, leading to diabetic-like conditions such as retinopathy and vascular dysfunction (Gleeson *et al*., 2007). Long-term exposure to high-calorie diets or glucose-rich environments induces insulin resistance and metabolic abnormalities, mirroring type 2 diabetes mellitus. Zebrafish models have shown decreased insulin receptor expression and elevated serum fructosamine levels following chronic glucose exposure (Toyoshima *et al*., 2008). Transgenic lines with tissue-specific insulin resistance (e.g., via IGF-I receptor overexpression or liver-targeted CRISPR knockdowns) have further expanded the scope for mechanistic studies and therapeutic screening in diabetes research (Ghaddar *et al*., 2022). Zebrafish thus serve as an efficient and scalable platform for metabolic research and antidiabetic drug discovery.

**2.5 Zebrafish as a Model for Nonalcoholic Fatty Liver Disease (NAFLD) and Liver Disorders**

The zebrafish (*Danio rerio*) has emerged as a vital model organism for studying liver diseases, including Nonalcoholic Fatty Liver Disease (NAFLD), due to its significant genetic and physiological homology with the human liver. The zebrafish liver shares key cellular and functional features with the human liver, facilitating the investigation of hepatic disorders and metabolic dysfunctions. Notably, zebrafish have been extensively utilized to model liver carcinogenesis, wherein exposure to carcinogens induces liver tumors that exhibit gene expression profiles similar to those found in human hepatocellular carcinoma (Patton *et al*., 2021). Experimental exposure to a 6% fructose solution causes hepatic steatosis in zebrafish, closely mimicking the fat accumulation characteristic of NAFLD in humans with high carbohydrate intake (Ferrari *et al*., 2018). Moreover, dietary overfeeding in zebrafish accelerates fatty liver development and liver carcinogenesis, accompanied by dysregulation of leptin—a hormone integral to metabolism and obesity mirroring mechanisms observed in human obesity-related liver disease (**Shimizu** *et al*., 2023).

**2.6 Zebrafish as a Model for Cardiotoxicity**

Cardiotoxicity represents a significant concern in drug development, and zebrafish serve as a robust in vivo model to evaluate cardiac safety. The physiological and molecular pathways underlying cardiotoxicity in zebrafish embryos closely resemble those in humans. For instance, treatment with drugs such as clomipramine and terfenadine in zebrafish induces cardiac dysfunction, hemorrhaging, edema, arrhythmias, and ultimately mortality, paralleling clinical cardiotoxic effects. Transgenic zebrafish models further facilitate the study of cardiac responses to small molecules, enabling detailed assessment of heart rate regulation and drug-induced cardiotoxicity at cellular and systemic levels (Patton *et al*., 2021).

**2.7 Zebrafish as a Model for Lipid-related Diseases**

Due to conserved lipid metabolism pathways, zebrafish are widely used to investigate lipid-related disorders such as atherosclerosis and obesity. Zebrafish exhibit comparable lipid absorption, processing, and regulation systems to mammals. They allow in vivo visualization of lipid deposition within blood vessels and macrophage lipid accumulation key contributors to atherosclerotic plaque formation. The melanocortin system, responsive to leptin, plays a crucial role in zebrafish energy homeostasis, mirroring mammalian fat regulation. Key transcriptional regulators of cholesterol metabolism, including sterol-regulatory element-binding proteins (SREBPs) and liver X receptors (LXRs), are conserved in zebrafish, with genetic mutations producing phenotypes analogous to human lipid disorders, thus supporting their use in obesity and cholesterol metabolism research (Holtta-Vuori *et al*., 2010).

**2.8 Zebrafish as a Model for Tumorigenesis**

Zebrafish provide an advantageous vertebrate model for tumor biology due to their genetic similarity to humans and capacity to develop tumors analogous to those in human patients. Their conserved tumor suppressor genes and oncogenes allow exploration of the genetic basis of cancer (Patton *et al*., 2021). Histopathological analyses reveal that chemically induced tumors in zebrafish closely resemble human tumors in cellular architecture and progression (**Wojciechowska** *et al*., 2016). Gene expression studies during tumor progression in zebrafish liver demonstrate conserved molecular signatures with human liver cancer, reinforcing the model’s relevance for understanding tumorigenesis and evaluating anticancer therapies (**Mudbhary** *et al*., 2011).

**2.9 Zebrafish as a Model for Kidney Disorders**

Zebrafish kidneys share important structural and functional traits with mammalian kidneys, particularly in osmoregulation and waste excretion, making them suitable for kidney disease research. Zebrafish models have been employed to study glomerular filtration, renal tubular function, polycystic kidney disease (PKD), nephronophthisis, and acute kidney injury (AKI). The transparency of zebrafish embryos enables real-time observation of kidney development and disease progression, facilitating insights into pathophysiology and therapeutic intervention. Furthermore, the genetic homology with humans supports investigations into the genetic basis of renal disorders and high-throughput screening of potential treatments (**Outtandy** *et al*., 2019).

**2.10 Zebrafish Models in Muscular Dystrophy Research**

Muscular dystrophies (MD) are characterized by progressive muscle degeneration and functional impairment. Zebrafish have become instrumental models for these disorders due to their genetic and physiological relevance. The sapje mutant zebrafish mimics Duchenne muscular dystrophy (DMD), displaying pathological features similar to affected humans. For myotonic dystrophy type 1 (DM1), zebrafish deficient in Muscleblind-like 2 (MBNL2) protein show phenotypes resembling human disease, highlighting RNA splicing defects central to DM1 pathogenesis (Machuca-Tzili *et al*., 2005). Additionally, zebrafish expressing expanded CUG repeats recapitulate transcriptional and developmental abnormalities characteristic of DM1 (Todd *et al*., 2014). Zebrafish models also help elucidate congenital muscular dystrophies, such as CMD Type 1A related to LAMA2 mutations, expanding understanding of laminin α2 deficiency (Jones *et al*., 2001). Their optical transparency and aquatic absorption of compounds facilitate efficient in vivo drug screening and pharmacological testing. Genome editing technologies like CRISPR/Cas9 have further enhanced zebrafish models, allowing precise replication of human mutations and accelerating therapeutic research (Hwang *et al*., 2013). Overall, zebrafish provide a cost-effective, scalable platform bridging basic muscular dystrophy research with clinical translation.

**3. CONCLUSION**

Zebrafish (*Danio rerio*) have firmly established themselves as a powerful and versatile platform for preclinical research. Their genetic and physiological similarity to humans, coupled with advantages such as optical transparency, rapid development, and amenability to high-throughput screening, make them ideal for modeling a broad spectrum of human diseases. From liver and cardiovascular disorders to metabolic syndromes, cancer, kidney diseases, and muscular dystrophies, zebrafish enable real-time, in vivo insights into disease mechanisms and therapeutic responses. Furthermore, their cost-effectiveness, ethical viability, and ease of maintenance position them as a valuable alternative to traditional mammalian models. As the demand for reliable, scalable, and translationally relevant models grows, zebrafish are poised to play an increasingly pivotal role in bridging the gap between basic research and clinical application in drug discovery and biomedical innovation.

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