Zebrafish (*Danio rerio*) as an Animal Model for Preclinical Research: A Comprehensive Review

Srinivas Nandyala [a*], Laxmikant B. Borse [a], Narhari G. Yedke [a], Pavani Gayatri Alluri [a] and M. Suresh Babu [b]

[a] *Department of Pharmacology, Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra- 422213, India.*
[b] *School of Pharmacy, GIET University, Gunupur, Odisha- 765022, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Review Article

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

_____

Corresponding author: Email: srnvs87akshi@gmail.com;

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.

Keywords: 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 (Song *et al.*, 2024). 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).

Fig. 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 (Bertho *et al.*, 2021). Adult zebrafish are relatively small, measuring between 4 and 5 centimetres in length (Allison *et al.*, 2009). 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 (Ochenkowska *et al.*, 2022). 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 (Sadamitsu *et al.*, 2024). 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 behavioural and physiological experiments, enhancing data reproducibility. These cumulative features underscore the zebrafish's pivotal role in advancing research across drug discovery, disease modelling, 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 modelling tumour development and progression (Dorner *et al.,* 2024). Forward and reverse genetic approaches in zebrafish have facilitated the identification of oncogenes, tumour 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 tumour 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 tumour 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 (*Danio rerio*) have emerged as a highly effective model for epilepsy research due to their significant neurological homology with humans and their suitability for genetic manipulation. Approximately 85% of human epilepsy-associated genes have homologs in zebrafish, enabling the creation of targeted genetic mutations to investigate the molecular basis of seizure susceptibility (Baraban *et al.,* 2013). This makes zebrafish particularly valuable for modeling monogenic forms of epilepsy and exploring genotype–phenotype relationships. Drug administration in zebrafish larvae is straightforward, as they readily absorb compounds from their surrounding aquatic environment. This facilitates rapid screening of candidate antiseizure drugs (ASDs) and neurotoxicity assessment. The transparent nature of zebrafish embryos and larvae further enhances real-time observation of neuronal activity and behavioral changes. Behavioral assays and electrophysiological recordings in zebrafish have been validated to replicate key features of human seizure activity, enabling high-throughput analyses of drug efficacy (Kearney *et al.,* 2018). Recent studies underscore the potential of zebrafish in bridging gaps left by classical rodent models. Although zebrafish possess a comparatively simpler brain structure, their utility in modeling central nervous system disorders such as epilepsy, Parkinson's disease, and Alzheimer's disease is well-documented. Seizures in zebrafish are typically induced using chemoconvulsants such as pentylenetetrazol (PTZ), kainic acid (KA), and pilocarpine, all of which generate reproducible seizure phenotypes. These models provide a platform not only for ASD screening but also for understanding epileptogenic mechanisms and network-level neuronal dysfunction (D'Amora *et al.,* 2023). The scalability, low maintenance cost, and reduced ethical concerns associated with zebrafish make them a practical alternative to traditional rodent

models, particularly in early-phase drug discovery. Moreover, zebrafish allow for large-scale genetic screens and longitudinal behavioral monitoring, thus serving as a robust platform for identifying novel therapeutic targets and evaluating seizure-modulating 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 remains a critical challenge in drug development, often leading to the discontinuation of promising therapeutic compounds during preclinical or clinical stages. Zebrafish have emerged as a powerful *In vivo* model for evaluating cardiotoxic effects due to their physiological and molecular cardiac pathways being highly conserved with humans (Bowley *et al.*, 2022). Exposure to cardiotoxic drugs such as clomipramine and terfenadine in zebrafish embryos has been shown to produce cardiac abnormalities including bradycardia, arrhythmias, pericardial edema, hemorrhage, and eventual lethality, which closely resemble adverse cardiac events seen in human patients. The transparent nature of zebrafish embryos allows real-time visualization of cardiac structure and function, making it possible to detect subtle morphological and functional abnormalities in response to drug exposure. Transgenic zebrafish lines expressing fluorescent markers in cardiac tissues enable precise tracking of myocardial development and damage. These models support quantitative assessments of heart rate, contractility, and blood flow under various experimental conditions (Patton *et al.*, 2021). Moreover, the introduction of genome editing technologies such as CRISPR/Cas9 has significantly advanced cardiotoxicity research in zebrafish. Targeted editing of genes associated with ion channel regulation, myocardial integrity, or oxidative stress pathways enables researchers to model specific cardiac pathologies and better understand the mechanisms underlying drug-induced cardiotoxicity. These genetic tools also facilitate high-throughput screening of cardioprotective agents, making zebrafish a valuable platform for both mechanistic and therapeutic exploration in cardiac safety pharmacology.

Table 1. Summary of zebrafish-based disease models in preclinical research

S.No	Disease/Condition	Zebrafish Model Used	Key Features / Outcomes	References
1	Vaccination and Immunization	Live infection models, fluorescent reporter strains	Host-pathogen interaction, immune cell dynamics, antigen response	Meijer *et al.*, (2011); Trede *et al.*, (2004); Bailone *et al.*, (2020); Lam *et al.*, (2004)
2	Cancer	Chemically induced tumors, transgenic & xenograft models	Tumorigenesis, drug screening, gene expression studies	Amatruda *et al.*, (2002); White *et al.*, (2008); Langenau *et al.*, 2003; Dorner *et al.*, (2024)
3	Epilepsy	PTZ-induced seizure model	Seizure behavior, neural activity monitoring, drug efficacy	Baraban *et al.*, (2013); Kearney *et al.*, (2018); D'Amora *et al.*, (2023)
4	Diabetes	STZ-induced hyperglycemia, high-glucose exposure	Pancreatic beta-cell damage, insulin resistance, glucose metabolism	Field *et al.*, (2003); Gleeson *et al.*, (2007); Toyoshima *et al.*, (2008); Ghaddar *et al.*, (2022)
5	Liver Diseases	High-fat diet, ethanol exposure, gene knockdown models	NAFLD, steatosis, liver regeneration, fibrosis	Patton *et al.*, (2021); Ferrari *et al.*, (2018); Shimizu *et al.*, (2023)
6	Cardiotoxicity	Drug-induced (e.g., terfenadine, clomipramine), transgenics	Edema, arrhythmias, heart rate changes, cardiac failure	Bowley *et al.*, (2022); Patton *et al.*, (2021)
7	Lipid-Related Disorders	High-fat diet, SREBP/LXR mutants, lipid staining	Atherosclerosis, obesity, lipid accumulation, macrophage infiltration	Holtta *et al.*, (2010); Ka *et al.*, (2021)
8	Tumorigenesis	Transgenic, chemical, and xenograft tumor models	Human-like tumor architecture, conserved oncogenes and suppressors	Patton *et al.*, (2021); Wojciechowska *et al.*, (2016); Mudbhary *et al.*, (2011); Astell *et al.*, (2020)
9	Kidney Disorders	PKD, AKI, nephronophthisis models, live imaging	Glomerular and tubular defects, nephron development, real-time imaging	Outtandy *et al.*, (2019); Anantharamu *et al.*, (2022)
10	Muscular Dystrophies	sapje (DMD), MBNL2 knockdown (DM1), CUG repeat expression, LAMA2 mutants	Muscle degeneration, splicing defects, congenital muscular dystrophy phenotypes	Tesoriero *et al.*, (2023); Machuca-Tzili *et al.*, (2005); Todd *et al.*, (2014); Jones *et al.*, (2001); Hwang *et al.*, (2013)

2.7 Zebrafish as a Model for Lipid-related Diseases

Zebrafish have emerged as a powerful model organism for exploring lipid-related disorders such as atherosclerosis, hyperlipidemia, and obesity, primarily due to the evolutionary conservation of lipid metabolism pathways shared with mammals (Ka *et al.*, 2021). Their lipid transport, absorption, and storage processes closely mirror those observed in humans, allowing researchers to simulate complex metabolic conditions with high fidelity. The optical transparency of zebrafish embryos and larvae facilitates live imaging of lipid accumulation within blood vessels, enabling visualization of early atherogenic events such as macrophage foam cell formation and plaque development. Key transcriptional regulators involved in lipid homeostasis—such as sterol regulatory element-binding proteins (SREBPs) and liver X receptors (LXRs)—are functionally conserved in zebrafish. Mutations or chemical modulation of these pathways result in phenotypes analogous to human lipid disorders, further validating their utility in cholesterol metabolism research (Holtta *et al.,* 2010). Additionally, zebrafish express a leptin-responsive melanocortin system that influences energy balance, body weight, and fat deposition, making them suitable for studying mechanisms underlying diet-induced obesity (Ka *et al.*, 2021). Moreover, zebrafish can rapidly develop hyperlipidemia upon exposure to high-fat diets or chemical inducers, enabling time-efficient evaluation of lipid-lowering drugs and dietary interventions (Huiting et al., 2015). Their small size and high fecundity support large-scale genetic or pharmacological screens for candidate genes or compounds affecting lipid regulation. Transgenic zebrafish lines with fluorescently labeled lipids or lipid-processing enzymes offer real-time, non-invasive insights into lipid dynamics in live animals. These collective features highlight the zebrafish model as a scalable, cost-effective, and translationally relevant system for investigating the etiology and treatment of dyslipidemia.

2.8 Zebrafish as a Model for Tumorigenesis

Zebrafish (*Danio rerio*) provide a highly effective vertebrate model for investigating tumorigenesis and metastatic processes due to their genetic similarity to humans, optical transparency, and ease of genetic manipulation. Key tumor suppressor genes, oncogenes, and signaling pathways involved in human cancers are conserved in zebrafish, enabling detailed studies of tumor initiation and progression (Patton *et al.*, 2021). Histological assessments of chemically induced tumors in zebrafish reveal striking parallels to human neoplasms in cellular morphology and tissue invasion patterns (Wojciechowska *et al.*, 2016). Zebrafish are particularly valuable for studying cancer metastasis, the leading cause of cancer-related mortality. Their transparent embryos and larvae permit real-time, high-resolution imaging of tumor–host interactions, cancer cell dissemination, angiogenesis, and microenvironmental dynamics (Astell *et al.*, 2020). Xenotransplantation of fluorescently labeled human cancer cells into immunologically permissive zebrafish larvae—whose adaptive immune system matures only after 28 days facilitates metastasis modeling without immunological rejection (Lam *et al.*, 2004). Additionally, gene expression studies in zebrafish tumor models reveal molecular signatures that are conserved with human cancers. For example, research on hepatocellular carcinoma in zebrafish has shown that the overexpression of *UHRF1* leads to the epigenetic silencing of tumor suppressor genes, mirroring mechanisms observed in human liver cancer (Mudbhary *et al.*, 2011). Such studies demonstrate the zebrafish's potential in both fundamental cancer biology and preclinical drug discovery. Transgenic models and CRISPR/Cas9 gene editing further enhance the utility of zebrafish in replicating specific oncogenic mutations and screening for anticancer compounds (Astell *et al.*, 2020). Collectively, these attributes position the zebrafish as a translational model for uncovering mechanisms of tumorigenesis and evaluating therapeutic efficacy across multiple cancer types.

2.9 Zebrafish as a Model for Kidney Disorders

Zebrafish have proven to be a valuable model for investigating renal diseases due to their anatomical and physiological similarities to mammalian kidneys, particularly in osmoregulation, excretion, and nephron structure. The zebrafish pronephros, the functional embryonic kidney, performs glomerular filtration and tubular reabsorption akin to the human nephron, making it ideal for studying

renal development and disease mechanisms (Anantharamu *et al.*, 2022). The optical transparency of zebrafish embryos enables real-time imaging of renal structures, facilitating detailed examination of glomerular injury, nephron patterning, and renal regeneration. Zebrafish models have been widely utilized to study conditions such as polycystic kidney disease (PKD), nephronophthisis, acute kidney injury (AKI), and chronic kidney disease (CKD). Genetic conservation between zebrafish and humans allows the modeling of inherited renal disorders and functional analysis of kidney-related genes. Tools such as CRISPR/Cas9 and morpholino antisense oligonucleotides are used to generate gene knockdown or knockout models for various nephropathies. Moreover, the small size, external development, and high fecundity of zebrafish enable high-throughput drug screening and nephrotoxicity assessment, supporting early-stage therapeutic discovery (Outtandy *et al.*, 2019; Anantharamu *et al.*, 2022).

2.10 Zebrafish Models in Muscular Dystrophy Research

Muscular dystrophies (MDs) are a heterogeneous group of inherited myopathies marked by progressive skeletal muscle degeneration, leading to loss of mobility and, in severe cases, cardiac or respiratory failure. Zebrafish (*Danio rerio*) have emerged as powerful and genetically tractable models for studying MDs due to their high genomic homology with humans, conserved muscle architecture, and suitability for *In vivo* imaging and high-throughput drug screening. Several zebrafish mutant lines recapitulate key clinical and molecular aspects of human MDs. The sapje mutant zebrafish lacks functional dystrophin and serves as a robust model for Duchenne muscular dystrophy (DMD), exhibiting muscle fiber degeneration, impaired motility, and early lethality features reminiscent of the human condition. Similarly, zebrafish deficient in Muscleblind-like 2 (MBNL2) protein display RNA splicing defects, mimicking myotonic dystrophy type 1 (DM1). Other transgenic zebrafish expressing expanded CUG repeats reproduce hallmark transcriptional and developmental defects of DM1 (Machuca-Tzili *et al.*, 2005; Todd *et al.*, 2014). For congenital muscular dystrophies (CMDs), including CMD Type 1A, zebrafish with mutations in LAMA2 reveal muscle detachment and laminin α2 deficiency similar to human phenotypes (Jones *et al.*, 2001).

Zebrafish models of MDs have been generated using diverse approaches, including ENU mutagenesis, morpholino knockdown, Tol2-based transgenesis, TALENs, and CRISPR/Cas9 genome editing. These models enable detailed studies of muscle fiber development, organization, and associated pathologies. Moreover, a variety of phenotyping assays—such as motor behavior testing, birefringence analysis, oxidative stress markers, and mitochondrial function assessments—have been developed to evaluate disease severity and progression. Innovative transgenic fluorescent biosensor lines, in which fluorescent reporters are driven by muscle-specific promoters or responsive elements, have provided real-time insights into molecular dynamics within individual muscle fibers. These biosensors enhance the capacity for non-invasive monitoring of disease progression and treatment response, facilitating high-throughput *In vivo* screening of pharmacological and genetic therapies. Overall, zebrafish models offer a scalable, cost-effective, and physiologically relevant platform for dissecting the molecular underpinnings of muscular dystrophies and accelerating translational research aimed at therapeutic discovery (Tesoriero *et al.*, 2023; Hwang *et al.*, 2013).

3. CONCLUSION

Zebrafish (*Danio rerio*) have firmly established themselves as a powerful and versatile platform for preclinical research, offering a unique combination of genetic relevance, physiological similarity to humans, and experimental tractability. Their optical transparency during embryonic and larval stages, rapid external development, and high fecundity allow for real-time visualization of developmental and pathological processes *In vivo*.

This model has demonstrated utility across a wide spectrum of human diseases. In neuroscience, zebrafish have proven highly effective in modeling epilepsy, Parkinson's disease, Alzheimer's disease, and other neurodegenerative conditions, enabling the study of neuronal activity, seizure susceptibility, and drug responsiveness. In oncology, transgenic zebrafish lines and xenograft models allow the exploration of tumorigenesis, angiogenesis, and drug screening with high precision. Similarly, zebrafish have contributed significantly to the study of metabolic disorders,

including obesity, diabetes, and lipid metabolism, due to their conserved pathways and responsiveness to dietary and pharmacological interventions.

Moreover, zebrafish are increasingly used in modeling cardiovascular diseases, congenital heart defects, and arrhythmias, thanks to their transparent vasculature and accessible heart function assays. In nephrology and hepatology, zebrafish offer tractable models for acute and chronic kidney diseases, as well as liver injury and regeneration studies. Muscle-related disorders, immune dysfunctions, and rare genetic syndromes have also been effectively studied using zebrafish, facilitated by gene-editing tools like CRISPR and morpholinos.

Beyond disease modeling, zebrafish support high-throughput drug screening, toxicology assessments, and phenotype-based discovery, making them particularly valuable in early-phase drug development. Their cost-effectiveness, ease of maintenance, and reduced ethical concerns further enhance their appeal compared to traditional mammalian models.

In conclusion, zebrafish stand out as a comprehensive, scalable, and translationally relevant model organism. Their broad applicability across diverse biomedical fields positions them at the forefront of preclinical research. As science advances toward precision medicine and personalized therapeutics, zebrafish are poised to play an increasingly pivotal role in bridging the gap between laboratory research and clinical innovation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Allison D'Costa., Shepherd, I. T. (2009). Zebrafish development and genetics: Introducing undergraduates to developmental biology and genetics in a large introductory laboratory class. *Zebrafish*, *6*(2), 169–177. https://doi.org/10.1089/zeb.2008.0562

Amatruda, J. F., Shepard, J. L., Stern, H. M., & Zon, L. I. (2002). Zebrafish as a cancer model system. *Cancer Cell*, *1*(3), 229–231. https://doi.org/10.1016/s1535-6108 (02)00052-1

Anantharamu, T. (2022). Role of zebrafish as an experimental model for renal disorders. *Zebrafish model for biomedical research* (pp. 81–92). Springer, Singapore. https://doi.org/10.1007/978-981-16-5217-2_5

Astell, K. R., & Sieger, D. (2020). Zebrafish *In vivo* models of cancer and metastasis. *Cold Spring Harbor Perspectives in Medicine,* *10*(8), a037077. https://doi.org/10.1101/cshperspect.a0370 77

Bailone, R. L., Fukushima, H. C. S., Fernandes, B. H. V., De Aguiar, L. K., Corrêa, T., Janke, H., *et al.* (2020). Zebrafish as an alternative animal model in human and animal vaccination research. *Laboratory Animal Research*, *36*, 13. https://doi.org/10.1186/s42826-020-00033-4

Baraban, S., Dinday, M., & Hortopan, G. (2013). Drug screening in *Scn1a* zebrafish mutant identifies clemizole as a potential Dravet syndrome treatment. *Nature Communications*, *4*, 2410. https://doi.org/10.1038/ncomms3410

Bertho, S., Kaufman, O. H., Lee, K. A., Santos-Ledo, A., Dellal, D., Marlow, F. L. (2021). A transgenic system for targeted ablation of reproductive and maternal-effect genes. *Development*, 148(13), dev198010. https://doi.org/10.1242/dev.198010

Bowley, G., Kugler, E., Wilkinson, R., Lawrie, A., van Eeden, F., Chico, T. J. A., *et al.* (2022). Zebrafish as a tractable model of human cardiovascular disease. *British Journal of Pharmacology*, 179(5), 900–917. https://doi.org/10.1111/bph.15473. PMID: 33788282

D'Amora, M., Galgani, A., Marchese, M., Tantussi, F., Faraguna, U., De Angelis *et al.* (2023). Zebrafish as an innovative tool for epilepsy modeling: State of the art and potential future directions. *International Journal of Molecular Sciences*, 24(9), 7702. https://doi.org/10.3390/ijms24097702

Dooley, K., & Zon, L. I. (2000). Zebrafish: A model system for the study of human disease. *Current Opinion in Genetics & Development*, *10*(3), 252–256. https://doi.org/10.1016/s0959-437x(00)00074-5

Dorner, L., Stratmann, B., Bader, L., Podobnik, M., Irion, U. (2024). Efficient genome editing using modified Cas9 proteins in zebrafish. *Biology Open*, 13(4), bio060401. https://doi.org/10.1242/bio.060401. PMID: 38545958, PMCID: PMC10997048

Ferrari, J. T., Ayres, R., Hammes, T. O., Silveira, T., & Uribe, C. (2018). Experimental model of hepatic steatosis by fructose in adult zebrafish: A pilot study. *Clinical and Biomedical Research*, 38(2). https://doi.org/10.4322/2357-9730.77997

Field, H. A., Ober, E. A., Roeser, T., & Stainier, D. Y. R. (2003). Formation of the digestive system in zebrafish. I. Liver morphogenesis. *Developmental Biology*, *253*(2), 279–290. https://doi.org/10.1016/s0012-1606(02)00017-9

Ghaddar, B., & Diotel, N. (2022). Zebrafish: A new promise to study the impact of metabolic disorders on the brain. *International Journal of Molecular Sciences*, *23*(10), 5372. https://doi.org/10.3390/ijms23105372

Gleeson, M., Connaughton, V., & Arneson, L. S. (2007). Induction of hyperglycaemia in zebrafish (*Danio rerio*) leads to morphological changes in the retina. *Acta Diabetologica*, *44*(3), 157–163. https://doi.org/10.1007/s00592-007-0257-3

Holtta-Vuori, M., Salo, V. T. V., Nyberg, L., Brackmann, C., Enejder, A., Panula, P., & Ikonen, E. (2010). Zebrafish: gaining popularity in lipid research. *Biochemical Journal*, 429(2), 235–242. https://doi.org/10.1042/BJ20100293

Howe K, Clark MD, Torroja CF, *et al.* (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, 496(7446), 498-503. https://doi.org/10.1038/nature12111

Huiting, L. N., Laroche, F. J. F., & Feng, H. (2015). The zebrafish as a tool to cancer drug discovery. *Austin Journal of Pharmacology and Therapeutics*, *3*(2), 1069. PMID: 26835511

Hwang WY, Fu Y, Reyon D, Maeder ML, Kaini P, Sander JD, *et al.* (2013) Heritable and Precise Zebrafish Genome Editing Using a CRISPR-Cas System. PLoS ONE 8(7): e68708. https://doi.org/10.1371/journal.pone.0068708

Hwang, W. Y., Fu, Y., Reyon, D., Maeder, M. L., Tsai, S. Q., Sander, J. D., Peterson, R. T., Yeh, J.-R. J., & Joung, J. K. (2013). Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature Biotechnology*, 31(3), 227–229. https://doi.org/10.1038/nbt.2501

Jones, K., Morgan, G., Johnston, H., Tobias, V., Ouvrier, R., Wilkinson, I., & North, K. (2001). The expanding phenotype of laminin α2 chain (merosin) abnormalities: case series and review. *Journal of Medical Genetics*, 38(10), 649–657. https://doi.org/10.1136/jmg.38.10.649

Ka, J., and Jin, S.W. (2021). Zebrafish as an emerging model for dyslipidemia and associated diseases. *Journal of Lipid and Atherosclerosis,* *10*(1), 42–56. https://doi.org/10.12997/jla.2021.10.1.42

Kalueff AV, Stewart AM, Gerlai R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in Pharmacological Sciences*, 35(2), 63-75. https://doi.org/10.1016/j.tips.2013.12.002

Kari, G., Rodeck, U., & Dicker, A. P. (2007). Zebrafish: An emerging model system for human disease and drug discovery. *Clinical Pharmacology & Therapeutics*, *82*(1), 70–80. https://doi.org/10.1038/sj.clpt.6100223

Kearney, J. A. (2018). Expanding the zebrafish toolkit for epilepsy research. *Epilepsy Currents*, *18*(1), 56–58. https://doi.org/10.5698/1535-7597.18.1.56

Lam, S. H., Chua, H. L., Gong, Z., Lam, T. J., & Sin, Y. M. (2004). Development and maturation of the immune system in zebrafish, *Danio rerio*: A gene expression profiling, *in situ* hybridization and immunological study. *Developmental and Comparative Immunology*, *28*(1), 9–28. https://doi.org/10.1016/s0145-305x(03)00103-4

Langenau, D. M., Traver, D., Ferrando, A. A., Kutok, J. L., Aster, J. C., Kanki, J. P., *et al.* (2003). Myc-induced T cell leukemia in transgenic zebrafish. *Science*, *299*(5608), 887–890. https://doi.org/10.1126/science.1080280

Lieschke, G. J., & Currie, P. D. (2007). Animal models of human disease: Zebrafish swim

into view. *Nature Reviews Genetics*, *8*(5), 353–367. https://doi.org/10.1038/nrg2091

Machuca-Tzili, L., Brook, D., & Hilton-Jones, D. (2005). Clinical and molecular aspects of the myotonic dystrophies: a review. *Muscle & Nerve*, 32(1), 1–18. https://doi.org/10.1002/mus.20301

McCluskey BM, Braasch I. Zebrafish phylogeny and taxonomy. In: The zebrafish in biomedical research. Academic Press; 2020. p. 15-24.

Meijer AH, Spaink HP. (2011). Host-pathogen interactions made transparent with the zebrafish model. *Current Drug Targets*, 12(7), 1000-1017. https://doi.org/10.2174/1389450117956778 01

Mudbhary, R., & Sadler, K. C. (2011). Epigenetics, development, and cancer: zebrafish make their mark. *Birth Defects Research Part C: Embryo Today*, 93(2), 194–203. https://doi.org/10.1002/bdrc.20207

Ochenkowska, K., Herold, A., & Samarut, É. (2022). Zebrafish is a powerful tool for precision medicine approaches to neurological disorders. *Frontiers in Molecular Neuroscience, 15*, 944693. https://doi.org/10.3389/fnmol.2022.944693

Outtandy, P., Russell, C., Kleta, R., & Bockenhauer, D. (2019). Zebrafish as a model for kidney function and disease. *Pediatric Nephrology*, 34(5), 751–762. https://doi.org/10.1007/s00467-018-3921-7

Patel, P., Nandi, A., Verma, S. K., Kaushik, N., Suar, M., Choi, E. H., *et al.* (2023). Zebrafish-based platform for emerging bio-contaminants and virus inactivation research. *Science of the Total Environment*, *872*, 162197. https://doi.org/10.1016/j.scitotenv.2023.162 197

Patton, E. E., Zon, L. I., & Langenau, D. M. (2021). Zebrafish disease models in drug discovery: From preclinical modelling to clinical trials. *Nature Reviews Drug Discovery*, *20*(8), 611–628. https://doi.org/10.1038/s41573-021-00210-8

Poleo, G. A., Denniston, R. S., Reggio, B. C., Godke, R. A., & Tiersch, T. R. (2001). Fertilization of eggs of zebrafish, *Danio rerio*, by intracytoplasmic sperm injection. *Biology of Reproduction*, 65(3), 961–966. https://doi.org/10.1095/biolreprod65.3.961

Sadamitsu, K., Velilla, F., Shinya, M., Tanaka, R., Ito, Y., Nakamura, H., *et al.* (2024).

Establishment of a zebrafish inbred strain, M-AB, capable of regular breeding and genetic manipulation. *Scientific Reports*, 14, 7455. https://doi.org/10.1038/s41598-024-57699-3

Shimizu, N., Shiraishi, H., & Hanada, T. (2023). Zebrafish as a useful model system for human liver disease. *Cells*, 12(18), 2246. https://doi.org/10.3390/cells12182246

Song, H., Shin, U., Nam, U., Kim, J., Lee, S., Park, Y., *et al.* (2024). Exploring hematopoiesis in zebrafish using forward genetic screening. *Experimental & Molecular Medicine*, 56, 51–58. https://doi.org/10.1038/s12276-023-01138-2

Teame T, Zhang Z, Ran C, Zhang H, Yang Y, Ding Q, *et al.* (2019). The use of zebrafish (*Danio rerio*) as biomedical models. *Animal Frontiers*, *9*(3), 68–77. https://doi.org/10.1093/af/vfz020

Tesoriero, C., Greco, F., Cannone, E., Ghirotto, F., Facchinello, N., Schiavone *et al.* (2023). *Modeling Human Muscular Dystrophies in Zebrafish: Mutant Lines, Transgenic Fluorescent Biosensors, and Phenotyping Assays*. International Journal of Molecular Sciences, 24(9), 8314. https://doi.org/10.3390/ijms24098314.

Todd PK, Oh SY, Krans A, He F, Sellier C, Frazer M, *et al.* (2014). CGG repeat-associated translation mediates neurodegeneration in fragile X tremor ataxia syndrome. *Neuron*, *78*(3), 440–455. https://doi.org/10.1016/j.neuron.2013.03.02 6

Toyoshima, Y., Monson, C., Duan, C., Wu, Y., Gao, C., Yakar, S., *et al.* (2008). The role of insulin receptor signaling in zebrafish embryogenesis. *Endocrinology*, *149*(12), 5996–6005. https://doi.org/10.1210/en.2008-0329

Trede, N. S., Langenau, D. M., Traver, D., Look, A. T., & Zon, L. I. (2004). The use of zebrafish to understand immunity. *Immunity*, *20*(4), 367–379. https://doi.org/10.1016/s1074-7613(04)00084-6

White, R. M., Sessa, A., Burke, C., Bowman, T., LeBlanc, J., Ceol, C., *et al.* (2008). Transparent adult zebrafish as a tool for *In vivo* transplantation analysis. *Cell Stem Cell*, *2*(2), 183–189. https://doi.org/10.1016/j.stem.2007.11.002

Wojciechowska, S., van Rooijen, E., Ceol, C., Patton, E. E., & White, R. M. (2016). Generation and analysis of

zebrafish melanoma models. *Methods in Cell Biology*, 134, 531–549. https://doi.org/10.1016/bs.mcb.2016.03.00 8

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/5040