Zebrafish in drug discovery:

The missing link in your preclinical pipeline

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The zebrafish *(Danio rerio)* is a small tropical freshwater fish, native to slow-moving streams and rice paddies of the southeastern Himalayan region. Easily recognized by its horizontal blue stripes, it typically reaches about 4 cm in length and lives two to three years under laboratory conditions. Over the past decades, it has become one of the most widely used vertebrate models in biomedical research.

Zebrafish share a high degree of genetic homology with humans and possess biological traits that align closely with modern research needs. They mature rapidly, reproduce in large numbers, and develop externally, producing hundreds of transparent embryos each week that allow real-time observation and large-scale testing. According to **EU Directive 2010/63, embryos are not considered protected animals until 5 days post-fertilization (dpf)**, which further enhances their ethical advantage in high-throughput applications and supports the 3Rs principle (Replacement, Reduction, and Refinement) by reducing reliance on mammalian models.

In addition, advanced gene-editing techniques such as CRISPR enable precise human disease modeling, making zebrafish a powerful platform for translational research. For biotech and pharma companies advancing drug discovery or building the pre-regulatory evidence package before entering costly Investigational New Drug (IND)-enabling studies, zebrafish provide physiologically relevant, ethically responsible, and scalable data that **accelerate decision-making** and help candidates reach the next value inflexion point.

1. Why your pipeline may be skipping a crucial step

The pharmaceutical industry faces a persistent bottleneck: too many promising preclinical candidates fail in clinical trials. On average, over 90% of drugs entering human studies do not reach the market, with lack of efficacy and unforeseen safety issues as the leading causes.

A major contributor to this problem is the **gap between *in vitro* reductionist systems and complex human physiology**. Rodent studies bring mammalian relevance but are slower, expensive, and low-throughput, and, alone, may fail to predict human responses, leading to costly late-stage attrition reliably.

Zebrafish close this gap by providing **whole-organism, vertebrate context** early in the pipeline, offering translational insights at high speed and relatively low cost. They allow the performance of multi-organ toxicity tests, disease-focused efficacy assays, or high-content phenotypic screens, generating actionable, physiologically relevant pre-regulatory evidence that complements the *in vitro* results and strengthens the package before moving into costly mammalian studies. In practice, zebrafish **help refine the preclinical decision-making process** and ensure that only the most promising compounds advance to the next value inflexion point.

2. What makes zebrafish a uniquely powerful model

Zebrafish offer a unique set of characteristics that make them particularly well-suited for translational research:

Genetic homology

Approximately 70 % of human genes have at least one zebrafish ortholog, and about 82 % of human disease-related genes are conserved.

Optical transparency

Their embryos are transparent, allowing direct observation of organ development, cellular interactions, and compound effects in real-time.

Whole-organism context

Unlike cell cultures, zebrafish provide a whole-organism context that captures systemic toxicity and efficacy, making results more predictive of human biology.

Cost-efficient and scalable

Zebrafish require minimal space and resources compared to mammalian models, and their small size and social nature allow compact, high-density housing that supports large-scale research at lower cost.

High reproductive capacity

With spawning every 10 days and 200-300 eggs per spawning, zebrafish provide large sample sizes that strengthen statistical power.

External fertilization and development

Eggs develop outside the mother, enabling easy administration of drugs (water incubation), straightforward gene editing, *in vitro* fertilization, and direct observation of early development.

Whole-organism studies in plate-based formats

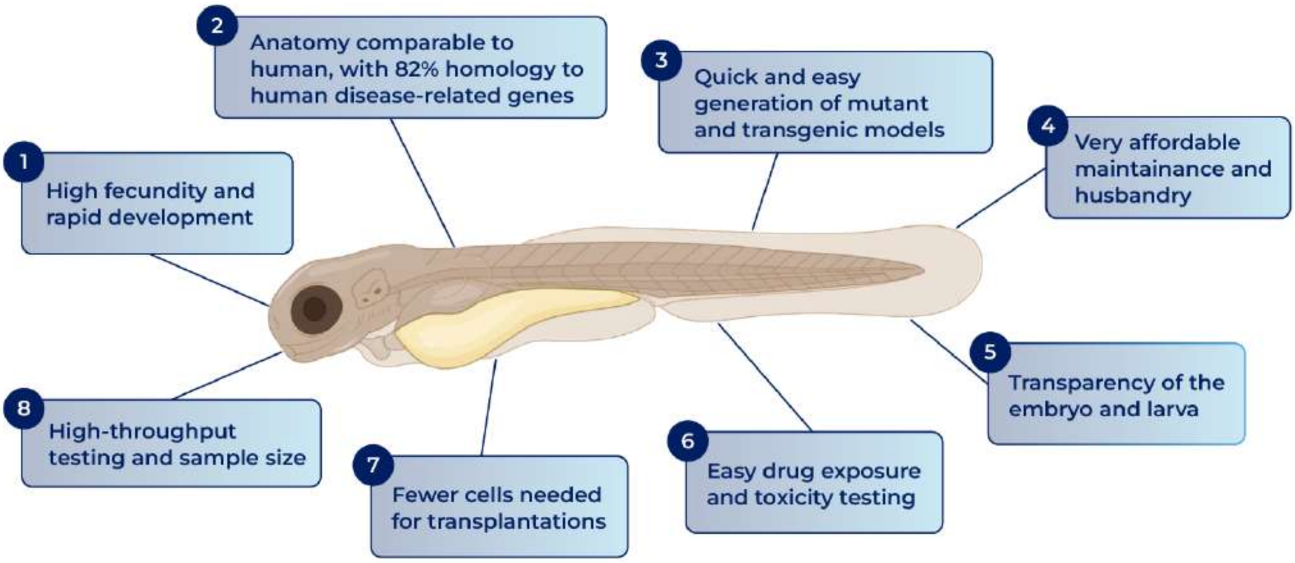
Embryos and larvae fit into multi-well plates, combining the throughput of *in vitro* assays with the complexity of a living vertebrate.

High-throughput screening

Their fecundity and small larval size make zebrafish ideal for testing large compound libraries in parallel.

Ethical alignment

The zebrafish model reduces dependence on mammalian studies and aligns with the 3Rs principle. Under EU Directive 2010/63, embryos remain outside the scope of animal protection legislation until 5 dpf, providing an additional ethical advantage for early, high-throughput experimentation.



Summary of the advantages of Danio rerio as an animal model in research. Adapted from: Fontana, C.M., Van Doan, H. Zebrafish xenograft as a tool for the study of colorectal cancer: a review. (2024). Cell Death Dis. 15:23.

Moreover, zebrafish develop rapidly (they reach major organ milestones within 72 hours) and externally, requiring minimal space and resources compared to rodents. Hundreds of embryos can be generated per mating, supporting large-scale phenotypic screens. Combined with genetic editing tools like CRISPR/Cas9 and advanced imaging systems, zebrafish enable precise, reproducible studies of disease mechanisms and drug responses. Their cost-efficiency and ethical advantage further strengthen their role, as they reduce reliance on mammalian models while delivering scalable, high-quality data.

This model is particularly useful in early efficacy, toxicity, and phenotypic validation studies. It allows researchers to test multiple compounds across varying doses and endpoints, accelerating decision-making and de-risking further development.

3. Why traditional models fall short on their own

In vitro models, including 2D and 3D cell cultures, allow for high specificity and controlled environments, but lack systemic complexity. Each of these models has strengths, but none offers a complete picture on its own. What the industry needs is a bridge, a model that retains the complexity of a whole organism while enabling medium- to high-throughput workflows, reducing costs and time while increasing the chances of translational success.

These are the advantages of zebrafish over traditional *in vitro* models:

Cell cultures

Traditional 2D cultures are cost-effective, controlled, and high-throughput, but they cannot fully replicate multicellular interactions, systemic metabolism, or immune responses. More advanced approaches, such as 3D cultures, organoids, and organ-on-a-chip systems, have improved tissue architecture, cell-cell communication, and certain metabolic or immune functions, yet they still fall short of reproducing the full physiological complexity of a living organism. Zebrafish address this gap by restoring systemic interactions in a vertebrate whole organism with high genetic homology to humans, making them particularly valuable for studies of development, toxicity, and disease mechanisms.

C. elegans

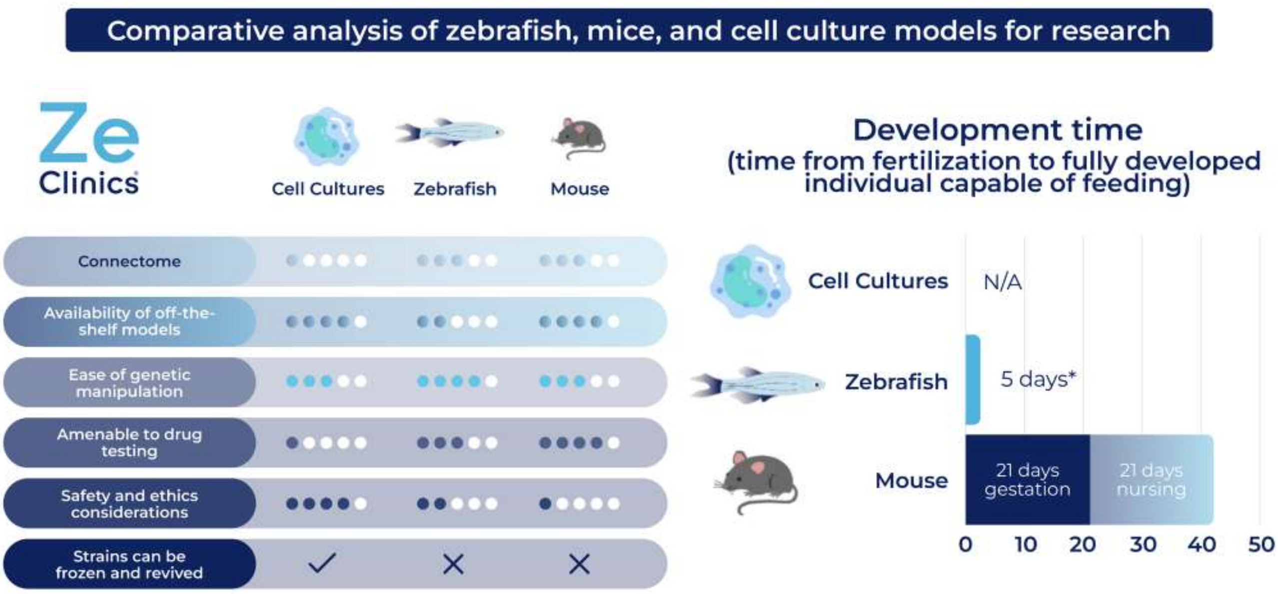
Provides genetic tractability and is cost-efficient, but with limited physiological similarity to humans. Zebrafish offer higher genetic homology and organ-level complexity while maintaining analogous throughput. Additionally, its transparency enables direct observation of embryonic development and organ function in real-time.

Drosophila

It is powerful for genetic screens, yet lacking many vertebrate-specific systems. Zebrafish bridge this gap with comparable screening capacity and vertebrate biology.

Rodents

Offer mammalian physiology, but breeding and testing are slow and costly. Zebrafish mature rapidly, reproduce in large numbers, and enable cost-effective large-scale screening with strong translational relevance.



What the industry needs is a complementary model that preserves the throughput and scalability of simpler systems while adding vertebrate complexity. Zebrafish provide that bridge: a cost-efficient, ethically favorable model that enables early, whole-organism data generation without the delays and expense of mammalian studies.

Platforms like our ZeGlobalTox, which simultaneously capture cardiac, hepatic, neurological, and developmental toxicities in zebrafish embryos, illustrate how this model turns systemic insight into reproducible, decision-ready evidence at a stage where traditional models cannot deliver.

4. From zebrafish to the clinic: real-world impact in drug development

Zebrafish have played a pivotal role in advancing several drug development programs now in clinical stages. Peer-reviewed studies showcase their utility in numerous fields by providing early phenotypic validation, toxicity profiling, and efficacy data translatable to humans.

Oncology

Zebrafish **xenograft models** allow implantation of human tumor cells into transparent larvae, enabling real-time monitoring of tumor growth, angiogenesis, and therapeutic response. Studies have shown concordance between regression observed in zebrafish xenografts and outcomes later confirmed in mammalian models and clinical settings. This approach has supported the development of compounds such as all-trans retinoic acid, which advanced into Phase II clinical trials for adenoid cystic carcinoma, positioning zebrafish as a powerful complement to rodent oncology studies. It has also been shown as a powerful tool to **generate patient avatars**, allowing the selection of the best treatment in a very short time.

Neurology

In epilepsy research, zebrafish have proven particularly impactful. The scn1lab mutant line mimics SCN1A-driven Dravet Syndrome, **exhibiting spontaneous seizures** and pharmacological responses comparable to human patients. Compounds such as clemizole and its derivatives were first identified in this model and have since progressed to clinical trials, illustrating how zebrafish can accelerate rare neurological disease programs.

Hematology

Zebrafish models have advanced the study of hematopoietic disorders, including anemia and thrombocytopenia. Their **conserved hematopoietic program** and transparent embryos enable visualization of blood cell development and targeted chemical screens. In conditions like Diamond–Blackfan anemia, zebrafish carrying rps19 mutations have helped validate therapeutic approaches. Notably, the antipsychotic trifluoperazine has been repurposed based on zebrafish findings and is now under clinical evaluation at Phase I.

Rare diseases

The **genetic tractability** of zebrafish allows for rapid modeling of orphan indications. For example, knockdown of the NANS gene reproduces developmental defects associated with N-acetylneuraminic acid storage disease, including brain and skeletal abnormalities. Supplementation with exogenous sialic acid partially rescued these phenotypes, providing proof-of-concept for therapeutic intervention. In addition, zebrafish models have supported the discovery of ALK2 inhibitors (dorsomorphin derivatives) for fibrodysplasia ossificans progressiva, which advanced to Phase I clinical testing.

Immunology and transplantation

Zebrafish have also been applied to immune-related conditions. For instance, ProHema (a PGE2 derivative) was evaluated in zebrafish for its role in modulating hematopoietic stem cell activity. It showed promising outcomes in enhancing engraftment, reducing graft-versus-host disease, and **supporting applications in leukemia**. Based on these findings, ProHema advanced to Phase II clinical trials.

Validated compounds in zebrafish that made it to clinical trials

ProHema (PGE2 derivate)

Indicated for leukaemia, graft versus host disease.

Clinical trials at Phase II (NCT01627314; NCT00890500).

ALK2 inhibitors (dorsomorphin derivatives)

Indicated for fibrodysplasia ossificans progressive.

Clinical trial at Phase I (ACTRN12619000319178).

Clemizole (EPX-100)

Indicated for Dravet syndrome.

Clinical trial at Phase II (NCT04462770).

Trifluoperazine

Indicated for blood disorders (Diamond–Blackfan anaemia)

Clinical trial at Phase I (NCT03966053).

All-trans retinoic acid

Indicated for adenoid cystic carcinoma.

Clinical trial at Phase II (NCT03999684).

ZeClinics

These advances show that zebrafish are not a peripheral option but a **proven translational model**. From oncology xenografts and neurological disease lines to hematology and rare metabolic disorders, zebrafish have consistently generated data that aligns with mammalian outcomes and informs clinical development. Their ability to **connect mechanistic insights with whole-organism effects** demonstrates their value as a bridge between discovery and regulatory preclinical requirements, ultimately reducing uncertainty as candidates move toward the clinic.

5. Where does zebrafish fit in your pipeline?

Zebrafish **bridge the gap between cell-based assays and in vivo mammalian studies**, enabling whole-organism evaluation of toxicity and therapeutic effects, and other key endpoints. Their rapid embryonic development, prolific reproduction, and low maintenance allow high-throughput, cost-efficient screening across multiple organ systems while reducing reliance on rodent models. By integrating zebrafish early, companies can achieve measurable gains, such as shortening timelines by generating functional data within days and cutting preclinical costs significantly compared to traditional models. Beyond efficiency, zebrafish provide decision-ready data that guides compound selection before committing to regulatory phases, reducing attrition and positioning assets for the next critical value inflexion point. This makes them a strategic addition to drug discovery pipelines, complementing existing models rather than replacing them.

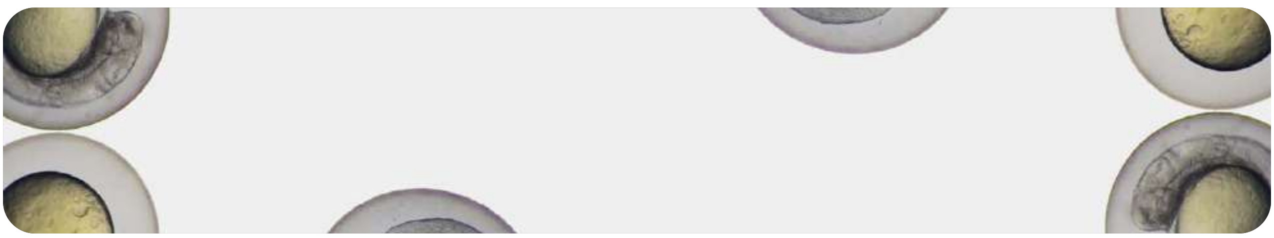
Reduce preclinical costs	Faster time-to-market	Enhance predictive success	Reduce Mammalian Animal Use
Up to 60% cost savings	Up to 40% time reduction	Early-stage zebrafish screening provides high-content functional insights	Narrowing down compounds transitioning to mammalian testing

Using zebrafish embryos, high-content, *in vivo* screening of large compound or gene libraries can be performed, thanks to their transparency and small size. This enables **rapid assessment of absorption, distribution, metabolism, excretion (ADME), toxicity, and therapeutic effects**, including organ-specific endpoints, within a living vertebrate system, all at a scale and cost far superior to traditional models.

Key applications include:

Disease model generation

Combining the use of CRISPR/Cas9 or tool compounds with easy phenotypic characterization enables the fast, cost-effective confirmation that a zebrafish disease model reliably reproduces a human pathology. Observable endpoints include morphological changes in organ structures and function, therapeutic responses in disease models, pathway-specific alterations, and behavioral shifts relevant to neurological research.



Target validation

Once a disease model is characterized phenotypically, the use of CRISPR/Cas9 allows evaluating the impact of gene disruption on disease worsening or recovery, as a mean for understanding its feasibility as a potential drug target. Interestingly, this type of target analysis can now be done at scale and in a very short time.

Efficacy studies

Evaluation of whether candidate compounds improve disease phenotypes using zebrafish models and high-throughput phenotypic screening.
This includes dose-finding, applying compounds to established disease models (e.g., models of epilepsy, Parkinson's, or non-alcoholic fatty liver disease (NAFLD)), and measuring whether treated fish recover wild-type characteristics, offering early insight into therapeutic potential within a living vertebrate context.

Toxicity testing

Zebrafish have proven valuable for predicting chemical substance toxicity. They allow the early evaluation of systemic and organ-specific toxic effects, including developmental (embryonic and organ formation), cardiac, hepatic, and neurological endpoints. Zebrafish assays can capture acute lethality, teratogenicity, and functional impairments within days, offering predictive insight before progressing to mammalian studies.

Integrating zebrafish into your workflow doesn't require an overhaul. It complements existing approaches and provides data that is physiologically relevant, reproducible, and actionable.

An integrative approach to refine early-stage drug screening

EARLY-STAGE PHASE | PRECLINICAL PHASE

In Vitro: CELL MODELS

In Vivo: ZEBRAFISH MODELS

In Vivo: MICE MODELS

6. Why your objections to using zebrafish do not hold up

While zebrafish have gained traction, some misconceptions persist. Let's address the most common concerns with facts:

"Zebrafish data will not integrate with our current workflow"

Zebrafish assays are highly customizable and can be aligned with your therapeutic area and pipeline goals. Whether you are focused on oncology, central nervous system, cardiovascular, or metabolic diseases, assays can be designed to match your endpoints.

"The upfront costs are too high"

Compared to rodent models, zebrafish are significantly more cost-efficient, especially at a large scale. Lower facility requirements, faster development times, and higher throughput translate to a lower cost-per-data-point.

"Regulators do not accept zebrafish"

Zebrafish data is increasingly being cited in regulatory submissions. While not yet a standalone model for IND filings, they provide valuable evidence as an orthogonal model, supporting safety and efficacy claims, and are often seen as strengthening the overall preclinical package.

"They are not predictive enough"

Studies have shown high concordance between zebrafish data and mammalian outcomes, especially for toxicity and efficacy endpoints. Their whole-organism context gives them an advantage over cell cultures, and their speed and scalability enhance decision-making.

Zebrafish have become an indispensable part of modern preclinical research. Their unique combination of genetic similarity, rapid development, transparency, and scalability fills a critical gap between cell-based assays and mammalian studies. By delivering whole-organism data earlier, zebrafish reduce late-stage attrition, uncover safety issues before they become costly setbacks, and provide actionable evidence to guide development strategies.

For drug discovery teams, regulatory-focused programs, and genetic research groups alike, zebrafish offer a proven way to accelerate timelines while enhancing scientific rigor. They **deliver translational, pre-regulatory datasets that de-risk portfolios, support smarter go/no-go decisions, and help advance candidates** to the next development milestone with stronger evidence. That is, they are not a replacement for existing models, but a bridge that strengthens the entire pipeline, from early phenotypic screens to efficacy and toxicity studies that inform regulatory submissions.

ZeClinics has translated these advantages into dedicated platforms that address specific preclinical needs:

- **ZeGenesis** applies advanced genetic tools to generate disease-relevant zebrafish models and perform target validation screenings
- **ZeEfficacy** assesses therapeutic potential by testing whether candidate compounds can rescue validated disease phenotypes
- **ZeTox** enables rapid and predictive systemic and organ-specific toxicity testing.

Together, these platforms provide physiologically relevant, reproducible data that reduce uncertainty, de-risk programs, and support regulatory milestones.

If your goal is to advance candidates with greater confidence, minimize risk, and improve translational success, integrating zebrafish into your workflow can make the difference. Explore the ZeClinics platforms or connect with our scientific team to discuss how zebrafish can support your next development milestone.

Let's talk about how zebrafish can support your next development milestone.

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